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ELEVENTH TECHNICAL ALFALFA CONFERENCE PROCEEDINGS

Held July 29 and 30, 1971, Albany, Calif.

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THE ELEVENTH TECHNICAL ALFALFA CONFERENCE reported here was held July 29 and 30, 1971, at the Western Regional Research Laboratory, Albany, Calif., headquarters of the Western Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture.

The meeting was cosponsored by the American Dehydrators Association and the U.S. Department of Agriculture. Attendance included certified seed growers, forage growers and processors, equipment manufacturers, agricultural consultants, State experiment station personnel, and members of government groups engaged in forage research.

The program was developed by E. M. Bickoff, J. Chrisman, and George O. Kohler of the Field Crops Laboratory, WMNRD, ARS, USDA, and Kenneth D. Smith, Executive Vice President, American Dehydrators Association.

The work discussed in the wet processing symposium was the result of a team effort by the following scientists: G. O. Kohler, E. M. Bickoff, Donald de Fremery, Mel E. Lazar, Benny E. Knuckles, Ray E. Miller, Sue C. Witt, Albert N. Booth, R. M. Saunders, M. A. Connor, Richard E. Edwards, and Donald D. Kuzmicky.

Statements made by participants do not necessarily represent views of the U.S. Department of Agriculture. Underscored numbers in parentheses refer to references at the end of each paper. The data, illustrations, and references are reproduced essentially as they were supplied by speakers.

This report was prepared in the Western Regional Research Laboratory, Berkeley, Calif. 94710. Copies are available on request.

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RESEARCH PROJECTS WITH DEHYDRATED ALFALFA

Kenneth D. Smith, Executive Vice President,
American Dehydrators Association, Kansas City, Mo.

This report summarizes much of the research conducted on dehydrated alfalfa since the 10th Technical Alfalfa Conference in 1968. Only brief abstracts of data are being presented. For further information concerning any of these projects, contact either the researcher or the American Dehydrators Association.

Value of Dehydrated Alfalfa in Horse Rations. L. H. Breuer, Jr., Texas Agricultural Experiment Station, Texas A&M University, College Station, Tex.

Three rations were compared. The basal ration; the basal with 20 percent dehydrated alfalfa replaced an equal amount of milo; and the basal with 40 percent dehy replaced 20 percent milo and 20 percent sorghum hay. Compared with the basal ration, one-half pound less of the 40 percent dehy ration was required to maintain a 1,000-lb. horse. Though not large enough to be statistically significant, dehy had a slightly higher energetic value for maintenance of horses than the 20 percent milo it replaced in the second ration and the 20 percent milo and 20 percent sorghum hay it replaced in the third ration.

These benefits were in addition to the usual advantages of dehydrated alfalfa due to its protein, vitamin, and mineral content, uniformity, quality, and superior handling characteristics as compared with conventional hay. Comparative feeding values of rations were as follows:

Ration	Daily amount (pounds) required to maintain 1000-lb. horse
Basal	13.18
20 percent dehydrated alfalfa	12.80
40 percent dehydrated alfalfa	12.59

Metabolizable Energy Value of Pelleted and Reground Dehydrated Alfalfa. Hobart R. Halloran, Halloran Research Farm, Inc., Modesto, Calif. and J. D. Summers, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada.

Evaluations of the metabolizable energy (M.E.) value of dehydrated alfalfa were made on laying hens (table 1) and chicks (table 2). The results of the chick test show that the metabolizable energy is markedly increased by pelleting. The hen test on three samples of reground pellets showed metabolizable energy values of 710-770 kcal./lb.

In Halloran's work with hens, feed intake and daily individual egg production were measured for 4 days. Droppings were collected and dried. Gross energy determinations were made on the diets and the dried feces. Chromium oxide was used as an inert tracer to measure the feed quantities consumed in relationship to the fecal output.

Table 1.--Summary of metabolizable energy value of 3 samples of dehydrated alfalfa meal

Sample	Pct. Protein	Metabolizable energy[1] kcal./lb.	
1 - - - - - - - - - - - -	20.3	770	740 quoted for 20 pct.
2 - - - - - - - - - - - -	21.7	710	
3 - - - - - - - - - - - -	25.3	730	

[1] 7 percent moisture basis.

In Summers' studies four different dehydrated alfalfa samples were tested with 3-week-old chicks to determine the effect of pelleting on metabolizable energy. The test diets were fed for 2 weeks and a 3-day fecal collection was made during the last week. Gross energy and nitrogens were determined on feed and feces samples and nitrogen-corrected M.E. values obtained.

Table 2.--Influence of steam pelleting on M.E. of dehydrated alfalfa

	Average weight		Average feed consumed		M.E.					
					Diet		Test ingredient[1]			
Ration	M	P	M	P	M	P	M.	P	M	P
	G.	G.	G.	G.		Kcal./g.			Kcal./lb.	
Basal- - - - - - -	326	333	542	600	2.73	2.70				
Basal +50 pct. (15 pct.)[2] - -	200	273	491	611	1.86	2.22	0.99	1.59	450	720
Basal +50 pct. (17 pct.)[2] - -	201	252	475	585	1.88	2.18	1.03	1.66	470	750
Basal +50 pct. (20 pct.)[2] - -	216	287	471	576	2.16	2.51	1.58	2.32	715	1050
Basal +50 pct. (22 pct.)[2] - -	217	281	473	620	2.09	2.22	1.44	1.76	650	800

[1] M = mash; P = pelleted. [2] Alfalfa samples supplied by WRRL, USDA, Albany, Calif.

Previous reports have demonstrated that high fiber ingredients could be improved by pelleting. While it is true that many poultry rations are being pelleted or crumbled and improvements in M.E. of such products as dehydrated alfalfa are already being achieved, these products are not being credited for this enhancement. This is because most of the M.E. values reported in the literature were derived from ingredients fed in the form of meal.

Phytate Phosphorous in Plant Ingredients. T. S. Nelson, L. W. Ferrara, and N. L. Storer - Poultry Science, XLVII, July 1968. Phytate phosphorous and phytic acid content of feed ingredients were as follows:

Ingredients specialty	Samples analyzed	Phytate phosphorous		Phytic acid percent[1]
		Percent	Percent of total phosphorous	
Alfalfa meal, 17 percent protein - - - - - - - - - -	6	<0.01	0	0
Corn gluten meal, 41 percent protein- - - -	6	0.35±0.09	60±15	1.2
Cornmeal, degermed- - - - -	15	0.07±0.02	69±16	0.3

[1] Dehydrated alfalfa contains no phytate phosphorous. The nonphytate phosphorous is considered equally as available as that in a good-quality, inorganic phosphate.

Dehy as an Extender of Urea in Dairy Cattle Rations. H. R. Conrad, J. W. Hibbs, and R. W. Wallenius, Ohio Agricultural Research and Development Center, Wooster, Ohio - 1969 Annual Report to American Dehydrators Association.

The results presented are those obtained with 11 Holstein cows in a switchover experiment. The experimental periods were for full lactation periods of 305 days.

A pelleted combination of 66 percent dehydrated alfalfa, 31.6 percent urea, 2 percent dicalcium phosphate, 0.6 percent sodium sulfate, and 0.4 percent sodium propionate (a preservative) was used as the protein supplement. It contained 16 percent total nitrogen (100 percent crude protein equivalent) and when added to cornmeal and ground oats at the 10 percent level resulted in a concentrate ration of 18-19 percent protein equivalent (3 percent urea). The concentrate fed in the control periods contained soybean meal on an isonitrogenous basis.

The following shows the average feed intake and lactation yields with standard deviation of the means.

Observation made	Dehy-100	Soybean meal
Number of lactations - - - - -	11	11
Total milk yield, pounds - - -	17,266 ± 1978	18,000 ± 2251
Milk protein, pounds - - - - -	530 ± 46	548 ± 84
Milk protein, percent- - - - -	3.06 ± 0.26	3.06 ± 0.27
Milk fat, pounds - - - - - - -	617 ± 71	607 ± 76
Milk fat, percent[1] - - - - - -	3.57 ± 0.41	3.37 ± 0.40
4 percent FCM, pounds- - - - -	16,145 ± 1771	16,313 ± 2039
Dry matter intake, pounds- - -	12,290 ± 1511	12,354 ± 1278
Digested dry matter, pounds- -	8,603 ± 1058	8,649 ± 899

[1] Differences between mean fat percentages are significant.

In work conducted over 4 years, milk yields of cows fed dehy and urea were equal to those receiving soybean meal as the protein supplement. High production was maintained with consumption up to 1 pound of urea and 2 pounds of dehydrated alfalfa daily and with 24 percent to 45 percent of the total nitrogen intake from urea. The fat percent was higher for cows fed dehy plus urea ($P < 0.05$).

Pelleted Dehydrated Alfalfa as a Supplement for Brood Mares.

James H. Bailey, D.V.M., Pampa, Tex.

Since 1966, brood mares have been fed increasing levels of dehy pellets in place of hay. The treatment mares were supplemented with 10 to 18 pounds per head per day beginning in November 1969 and continuing through the 1970 breeding season. Due to severe drought, six of the treatment mares were fed 20 pounds per head per day of dehydrated alfalfa through the summer and fall. Foaling and conception rates were compared with previous year's records of the same mares, and with records from "outside" mares of unknown nutritional background, which were used as the controls.

As the level of dehy increased in the treatment mares (as shown in following tabulation), the number of heat periods required per conception decreased. The treatment mares also had fewer heat periods per conception when compared with the control mares. It was observed that the foals from mares receiving dehy were much above average physical condition with no contracted tendons, crooked legs, or other abnormalities.

	1966	1967	1968	1969	1970
Treatment					
Number of mares	13	15	19	22	24
Number of heat periods	25	26	29	31	31
Number of breedings for conception	1.92	1.73	1.53	1.4	1.29
Control					
Number of mares	62	60	68	70	45
Number of heat periods	111	108	136	130	81
Number of breedings for conception	1.78	1.86	2.00	1.86	1.81

These demonstration trials show that brood mares benefit from adding dehydrated alfalfa to their ration and refute the myth that horses will not consume large amounts of dehy.

Dehy-100 Carries Urea Better Than Milo Urea Mixture. F. G. Owen and R. D. Appleman, University of Nebraska, Lincoln, Nebr. - Feedstuffs, February 6, 1971.

An experiment was conducted to compare a gelatinized milo-urea mixture (MUM), produced by extrusion dehy-100, and natural urea as sources of nitrogen in rations for lactating cows.

A 3 X 2 factorial arrangement of a randomized block design was utilized. Pre-experimental and postexperimental control periods were used to reduce error variance. The plan involved 36 Holstein cows assigned simultaneously to treatments. Cows were individually fed, ad libitum, complete rations consisting of a 3:1 ratio of corn silage (39 percent dry matter) to concentrate ration. The concentrate rations (21 percent crude protein) consisted principally of sorghum grain and soybean meal. The control ration contained 2.5 percent urea. For the other treatments, MUM or dehy-100 provided equivalent levels of nitrogen.

Daily milk yields were improved ($P < 5$ percent) by MUM and dehy-100 preparations of urea. Mean daily milk yields were 20.9 kg. for the control ration, 22.4 kg. for the dehy-100 ration, and 21.6 kg. for the MUM ration. Both dry matter and estimated net energy intakes were higher for the processed-urea rations. Effects of rations on milk fat and solids not fat content were small. However, dehy-100 was superior to MUM in support of body weight gain.

Dehydrated Lucerne (Alfalfa) in the Rations of Breeding Sows. Mr. D. Fish, Greenways Farm, Harlow, Essex collaborated with the U.S. Feed Grains Council in a practical trial on his farm.

His breeding herd during the period the trial consisted of 45 large white commercial breeding sows housed indoors on concrete for their entire breeding lives. The herd was randomly divided into two groups - control and experimental.

The diets were fed dry in meal form and mixed on the farm. A proprietary 50 percent crude protein/vitamin/mineral concentrate (fully fortified with 40 million International Units (m.i.u.) of vitamin A and 10 m.i.u. of vitamin D per long ton was fed with barley at a ratio of 1:9 for the dry-sow ration and 1:5.66 for sows with litter.

In the experimental ration 7-1/2 percent of cereals was replaced by 17 percent protein dehydrated lucerne (alfalfa) meal. No dried green chops were included in the control ration.

In the lucerne (dehydrated alfalfa) group there were the following increases over the control.

3 percent more piglets born
6.3 percent more reared to 3 weeks
4 percent more weaned at 6 weeks of age
6.7 percent more weight per litter at 6 weeks

Dehydrated Alfalfa in Complete Pelleted or Wafered Rations for Lactating Cows. F. G. Owen and R. D. Appleman, University of Nebraska, Lincoln, Nebr.

Progress is being made toward developing complete pelleted or wafered rations for self-feeding to the milking herd. Studies over the past 2 years have involved wafers (1-1/2 inches X 1-1/2 inches X 5/8 inch) and pellet (3/8-inch) mixtures. Dehydrated coarse-chopped alfalfa and whole corn plant have been used as roughage sources. The complete feed wafers contained both the roughage and grain in one preparation. The complete pellet rations were mixture of roughage pellets and grain pellets.

Feed consumption of these rations was excellent. Intakes were generally between 40 and 50 pounds daily. Consumption of pelleted feeds was equal to or higher than that for wafers. No off-feed or other digestive upsets were encountered. The value of such rations for maintaining milk yield appears to be very good to excellent. The problem with these rations is depression of milk fat percentage.

The water preparation was somewhat superior to pellets in maintaining milk fat. Rations with bentonite seemed to offer some benefit. Rations with 1 percent bentonite and 35 percent or less of grain supported a fat test of 3.4 to 3.5 percent.

Dehydrated Alfalfa Meal in Rations for Pigs and Brood Sows in Confinement. R. W. Seerley and R. C. Wahlstrom, South Dakota State University, Technical Bulletin 32, June 1968.

The experimental rations were fed to swine in total confinement on concrete floors. Young pigs (Hampshire, Duroc, and Spotted breeds) were started at 8 to 9 weeks of age, weighing from 31 to 49.5 pounds each. The calculated chemical composition of the rations was: Protein 16.0 percent; calcium 0.60 - 0.70 percent; and phosphorous 0.49 - 0.58 percent.

Summary of Growing and Finishing Swine Observations

Approximately 200 pigs were used in four trials. Rations were fed in meal and pellet form. The pelleted rations tended to produce greater daily gains and were used more efficiently than the same rations in mash or meal form. With 10 percent dehydrated alfalfa in the total ration there was a decrease in daily gain and feed efficiency.

With 5 percent dehydrated alfalfa in the pelleted total ration, the daily gain and feed efficiency were equal to that of the control ration, but in meal form there was a decrease in feed efficiency with the lower starting weight pigs (37.5 - 41 pounds) but not with the pigs that weighed 49.5 pounds at the start.

The pigs in trial 2, started at 49.5 pounds, performed as well with 10 percent dehydrated alfalfa as the controls or lower levels of dehydrated alfalfa. There was considerable variation in the feed intake and growth responses. This was probably due to the small number of pigs (6) per treatment.

Summary of Four Trials with Brood Sows

Comparisons and Observations

Trial 1 - 0, 2.5, 5, and 10 percent dehydrated alfalfa. A higher percentage of the sows fed 5 and 10 percent dehydrated alfalfa farrowed than of the sows fed 0 and 2.5 percent dehydrated alfalfa. Sows fed 10 percent dehydrated alfalfa weaned significantly more pigs than those fed the other levels of dehydrated alfalfa meal. Average litter weight was greatest for sows fed 10 percent dehydrated alfalfa meal.

Trial 2 - 14 and 18 percent protein in total ration with dehydrated alfalfa at 0, 2.5, 5, and 10 percent.
The higher level of protein (18 percent) increased the litter size of the sows fed the ration with no dehydrated alfalfa. The level of dehydrated alfalfa and protein had no significant effect on number of sows farrowing, birth weight, or other criteria. There were only six sows per lot.

Trial 3 - 0, 10, and 20 percent dehydrated alfalfa plus a ration with 10 percent dehydrated alfalfa with added protein, minerals, and vitamins.
Sows fed 20 percent dehydrated alfalfa produced significantly heavier pigs at birth and weaning than the other treatments. Litter size was significantly larger at weaning in the group fed 10 percent dehydrated alfalfa fortified with protein, minerals, and vitamins than the other 10 percent dehydrated alfalfa treatment.

Trial 4 - 0 and 10 percent dehydrated alfalfa.
There were no significant differences between treatments.

Some research projects have not been included in this report because they will be covered by separate speakers later on in the program.

Should you desire to be kept abreast of research conducted on dehydrated alfalfa, write to the American Dehydrators Association, 800 West 47th Street, Kansas City, Mo. 64112, and request to be placed on the research mailing list.

INTRODUCTION TO WET PROCESSING SYMPOSIUM

E. M. Bickoff
Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA, Berkeley, Calif.

Green leaves are the world's largest source of protein. Although this protein has excellent nutritional quality, leaves have limited use for monogastric animals because of the presence of indigestible fiber. Scientists have long known that crushing or grinding green plant material to break the cell walls yields a juice containing a high percentage of the plant protein.

This laboratory has been interested in this process for many years and basic steps were reported in Chemurgic Digest in 1947. They consisted of grinding the fresh alfalfa through a disintegrator to break down the cell walls, expressing the liberated juice from the ground tissues by hydraulic pressure, heating the press juice to coagulate the protein, separating the curd from the brown whey-like juice, and drying the curd to yield the leaf protein concentrate containing no fiber and 40 percent or more of protein. However, more recently the development of high-energy poultry rations has been reducing the market for high-fiber products such as dehydrated alfalfa meal. Therefore, a large potential market exists for a low-fiber, high-xanthophyll supplement for broilers and laying hens which can be fulfilled by leaf-protein concentrate. The green color of the chlorophyll will enhance rather than detract from the market value of the product for chick feed. Dehydrated alfalfa meal can be prepared from the pressed cake to meet the present market grades. Wet fractionation is easily incorporated into a conventional dehydrated alfalfa meal production process.

The leaf protein concentrate is then a byproduct of an already established process, rather than the primary product. Commercial development based on this approach has been undertaken by a cooperating commercial dehydrator (Batley-Janss Enterprises, Brawley, California). Their first plant is in commercial production manufacturing about 4,000 tons of leaf protein concentrate per year.

In the following symposium on wet fractionation the various unit processes involved are discussed in detail. The yield and composition of the various products are summarized. Studies on both in vitro and in vivo digestibility of the concentrate and stability of the pigments during storage are described.

REVIEW OF LEAF PROTEIN CONCENTRATE--PRODUCTION AND PROJECTED USES

G. O. Kohler
Western Marketing and Nutrition Research Division
Agricultural Research Service
U.S. Department of Agriculture
Berkeley, Calif.

At the 10th Technical Alfalfa Conference held in Reno several years ago, I described the project we had undertaken in collaboration with Batley-Janss Enterprises, Inc. (BJE), which has resulted in the establishment of an alfalfa wet processing plant. Our research and process involved will be covered by the film and several speakers who follow. My plan is to present a fairly broad picture of research on, or closely related to, leaf protein in different parts of the world.

First, I shall describe some of the processes which have been developed, and, second, I should like to describe work and objectives of some of the foreign workers in the field. In doing this I shall draw upon the published literature but more so upon the discussions at the Technical Group Meeting on Leaf Protein[1] which I attended in Coimbatore, India, in November 1970 and discussions with visitors from many parts of the world to our laboratory here in Albany (Berkeley). Since much of the research being done is directed toward production of human food grade leaf protein concentrate (LPC), a considerable part of my discussion will be on this subject.

At this point let me insert a comment on the India meeting. Attendance was by invitation. The chairman of the leaf protein working group is Dr. N. W. Pirie of Rothamsted, England, who has been working in the area of leaf protein since about 1940. Incidentally Mel Bickoff and I were working in this field at the same time. While we dropped LPC as impractical in the United States at that time (1940's) Dr. Pirie has been constant in his efforts to produce cheap human food from leaves for the last 30 years. At the Coimbatore meeting many of the participants were his ex-students who have continued their thesis work on returning to their home countries.

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[1] Sponsored and financed by International Biological Programme (IBP) Central Office, 7 Marylebone Rd., London, N.W. 1, U.K.

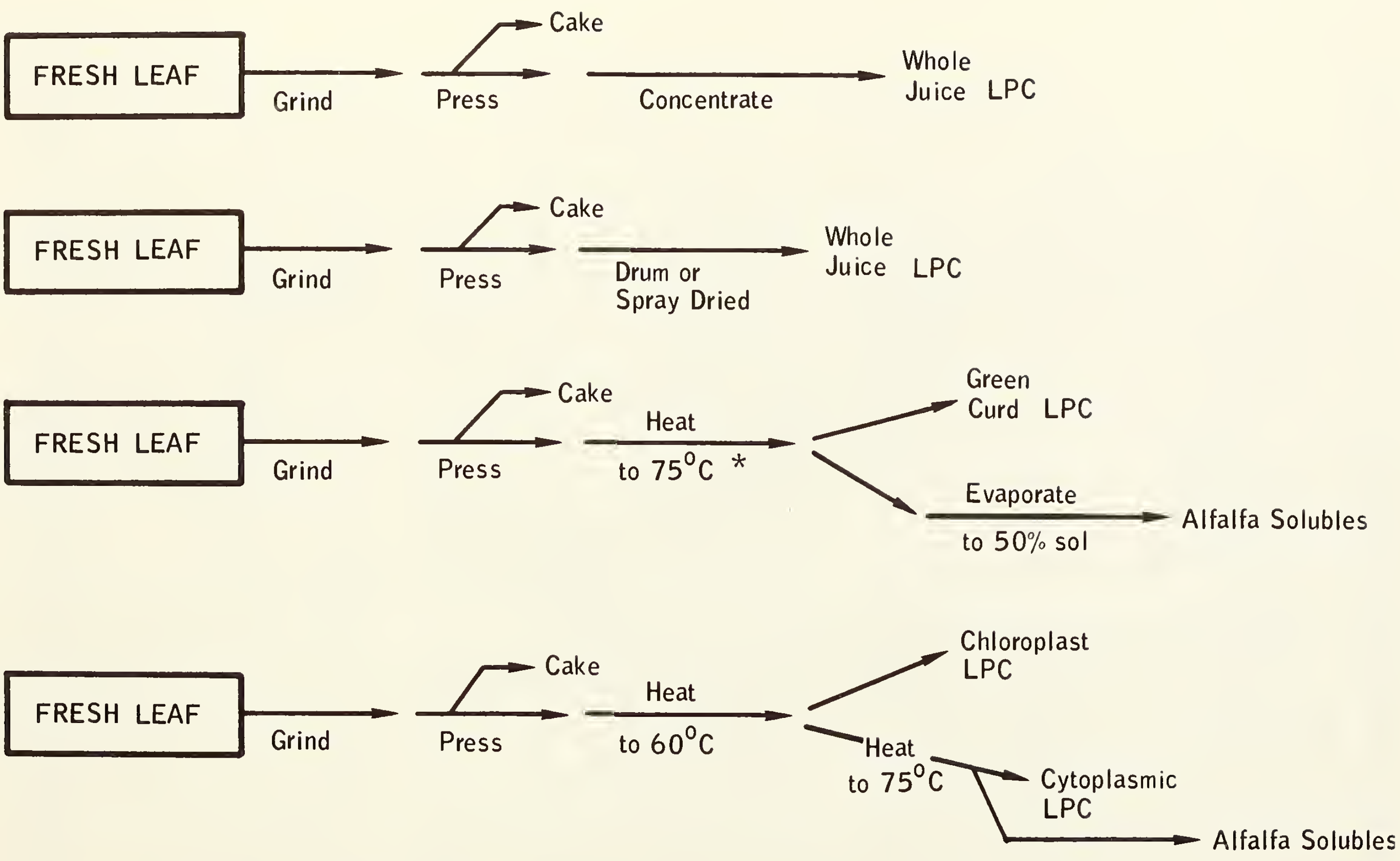
General Description of Processes for Making LPC

Figure 1 shows a number of processes which can be used to produce LPC. The simplest process is to grind and press the fresh leaf to produce a juice and a residue. The juice, which contains soluble leaf components as well as suspended chloroplasts and other cell parts, can be concentrated, dried, or even used as is if storage stability is not necessary. This type of material may be called "whole juice LPC" since it contains all of the salts, sugars, nonprotein nitrogen compounds, as well as such potentially deleterious compounds as alkaloids, cyanogenetic glycosides, goiterogens, saponins, etc. Such a whole juice LPC contains about 30-35 percent of crude protein on a dry basis.

If the green press juice is heated to 70° C.or over, a green curd is produced which can be separated from the brown wheylike liquid by decantation, filtration, or centrifugation. By making this separation much of the soluble nonprotein substances are separated from the protein. The latter may be dried or otherwise preserved to produce a product containing 40 to 50 percent crude protein on a dry basis. At WRRL we had as an objective for our Phase I work the production of a xanthophyll protein concentrate for use in poultry rations. Xanthophyll which is not soluble in water is present in high concentration in the juice since it is a component of the suspended chloroplasts which are removed with the juice. When the protein is coagulated by heat, the chloroplastic materials are carried down in the curd along with the protein. However, we have found that large losses of xanthophyll may occur during processing due to the action of the enzyme lipoxidase and air. We found if the pH of the juice were controlled at pH 8 to 8.5 by adding ammonia prior to juicing that this enzyme is not active so that high levels of xanthophyll become possible.

Addition of ammonia has additional advantages of reducing protease activity, of improving hardness of the curd formed on heating, and on stabilizing the green color of the product. The latter is considered a desirable factor in feed products. The dried LPC produced by this procedure we have named PRO-XAN. This is the product sold by BJE under the trade name "X-PRO."

Another modification of process #3 on figure 1 is used by Pirie and his coworkers. The modification consists in washing the green curd with dilute acid solution to remove the water soluble materials trapped in the curd. The acid assists in lowering ash content, in carrying out the washing step, and in reducing slightly the greenness



* Alternative methods of separating protein from water soluble fraction include, acidification to pH 4.0-4.5, allowing to stand at room temperature, precipitation with solvents, freezing and combinations of these.

Figure 1.--Several processes for preparing LPC and byproducts.

of the product. The products of the Pirie process are designed for human food, and greenness is not considered desirable in this case. The product may be stored as a pressed curd under refrigeration or may be dried and ground to form an olive-green powder containing about 55 to 65 percent protein, depending on the plant material from which it was made. At the Coimbatore meeting, the Working Group went on record to the effect that LPC should contain 1 percent or less of soluble solids, the object being to avoid the possible effects of deleterious components in the solubles and to raise the protein content to as high a level as possible.

The processes discussed thus far have been carried out on a pilot-plant scale and in the case of the PRO-XAN (or X-PRO) process, on a large commercial scale. All of these processes yield dark-colored products with relatively strong flavors. While Dr. Pirie and some of his associates feel that acceptance of the acid-washed product as a food can be accomplished, I believe that there is general agreement that if a white, tasteless product could be produced economically, it would gain quicker and wider acceptance. I will come back to this problem later.

In our opinion such a product will be essential for broad acceptance in the United States of products containing LPC. For convenience in discussion I shall define such a product as LPCW even though the white in some cases may be tinged with grey or tan. It follows that LPCG is green in color.

There are two laboratory procedures known for making LPCW. The most obvious is by extraction of LPCG from process 3 (either PRO-XAN or Pirie modification) with a solvent such as aqueous acetone, aqueous alcohol, etc. Work along these lines has been done by many workers including Dr. Pirie's group, Dr. Guha, Dr. Singh, Dr. Stahmann at the University of Wisconsin, and our own group. The extraction may be carried out either on the dried product or on the wet green curd product using a process similar to that used to prepare fish protein concentrate. The protein products are much improved in color and flavor and the extract can be recovered as a xanthophyll-carotene concentrate. A second possible procedure for obtaining a white leaf protein is by fractionation. It has long been known that the chlorophyll, xanthophyll, carotene, and other fatty materials of leaves are contained in tiny bodies in the leaf cells called chloroplasts. These chloroplasts are suspended in the liquid cell contents which contain a colorless protein mixture. The colorless protein has been designated as "cytoplasmic protein" although a part of it comes from broken chloroplasts.

If the green juice is centrifuged in the laboratory at a very high speed, the chloroplasts are thrown to the bottom of the tubes and the brown clear liquid above still contains the chlorophyll-free protein. If this liquid is heated or otherwise treated to coagulate the protein, it can be recovered, washed and if desired, dried to yield LPCW. This LPCW has even higher nutrition value than has the conventional LPC. These steps can be carried out in the laboratory, but much research remains to be done before we decide which procedures to use and get the system optimized and in pilot-plant production. Obviously less edible protein is obtainable by fractionation than by extraction of lipids.

For these reasons we are not now in a position to answer many questions concerning cytoplasmic protein separations.

So much for the processes which are developed or are under investigation. Let me turn now to more specific work being carried out in different laboratories.

Process Research Locations

The following are centers of research on the village-type plant which has been the subject of much of the work of the Rothamsted group:

Process research for small-scale operation for developing countries:

England: Pirie; Davys, Morrison, Festenstein (Rothamsted)
Pakistan: Shah (Jaipur)
India: Singh (Mysore)
Portugal: de Oliveira (Lisbon)
Brazil: Sampaio (Sao Paula)

I have already described the process briefly. The product is a green cheeselike or dried product which is intended for incorporation into the foods of persons in need of increased protein in their diets. It was felt that village-scale plants, in India, for example, would have to be subsidized by the government and that the production would be used locally probably pointing at young children as the first target group. I shall have a little more to say about acceptability of this "standard type" LPC later.

In another area of LPC research, large-scale sophisticated plants (see the following list) are the objective.

Process research for large-scale operation to produce feed or food:

U.S.A. Kohler, Bickoff, et al., (Calif.); Stahmann (Wis.); Kinsella (Cornell); Stovall (Texas).
New Zealand: Allison (Christchurch).
Hungary: Istvan, Hollo, Koch (Budapest).
Sweden: Tear (Tumba).
Great Britain: Chayen.
USSR: Fomin (Rostov-on-Don).

These would produce feed or food products competitive in a relatively free market. Our own work falls in this category. There is strong interest in this approach in New Zealand and Australia, where forages can be produced at very low cost and products possibly exported. In Hungary, interest in large-scale low-cost LPC is based on the high cost of soybean meal. If LPC could replace soybean meal in poultry and swine rations less soybean meal would have to be imported. In Sweden process research is being carried out largely by the Alfa-Laval Company which is, of course, interested in selling equipment. In the USSR interest is in developing means of preserving and recovering products from very high moisture forage crops.

Suitability of Different Leaves as Raw Material for Preparation of LPC

Research is being carried on at the following places:

Ceylon: Guneteleke (Colombo).
India: Joshi (Aurangibad); Matai (Calcutta).
N. Perur (Bangalore).
Pakistan: Shah (Jaipur).
Sweden: Lexander (Upsala).
Nigeria: Oke (Univ. of IFE).
U.S.A.: Stovall (Texas); Stahmann (Wis.).

The first category is the investigation of the processability of a wide variety of leaves. What is being determined is yield of protein per acre, yield of protein recovered through a standard extraction procedure, composition and flavor of the product, and effect of stage of growth, fertilizers, etc. on these parameters. Some leaves, such as corn, gave low extractability, but the leaves could be obtained cheaply as byproducts. Legumes gave good extractability in general. Some vegetable leaves gave excellent extractability (e.g., spinach), and in some cases the leaves are partly wasted in ordinary practice.

Amaranthus candatus was one of the best leaf crops studied from an overall standpoint. Alfalfa LPC was in the upper range from an extraction yield and quality standpoint, but according to Dr. Pirie it tended to be stronger in flavor than Amaranthus and some other LPC products. From our viewpoint here at WRRL alfalfa is the most practical source for most parts of the United States since agronomy, harvesting, handling, etc. have all been developed to a high level for on-the-farm use and for dehydration.

Product Evaluation Investigations.

Chemical and animal tests - all of the groups mentioned.

Human acceptability and nutritional studies:

India - Devadas (Coimbatore); Singh (Mysore).
Nigeria - Oke (Univ. of IFE).
West Indies - Waterlow (Jamaica).
Great Britain - Rothamsted group.
U.S.A. - Kohler, Bickoff, et al. (Calif.).

All of the workers mentioned above have done a great deal of analytical research on nutrients of LPC and nutritive value as determined by using laboratory animals. While attending the Coimbatore meeting we were given the opportunity of observing some small children come into the college while the meeting was held to get their rations of LPC as part of a study in progress by Dr. Devadas. Dried LPC from CFTRI in Mysore (Singh) was incorporated into a mixture of 10 percent LPC + 15 percent sugar + 75 percent of a grain called "ragi." Enough moisture had been added to make a fairly hard doughy mass which was formed into a ball about the size of a tennis ball. The children came in the morning and chomped away at their LPC ball for about 15 to 20 minutes, coming up with green mouths, cheeks, and chins.

In this form, LPC is apparently acceptable to hungry Indian children. Dr. Devadas has been carrying on studies on adults on LPC acceptance in various Indian dishes for about 4 years. In all cases, acceptance scores were decreased by LPC. At the 5-gram level the dhal balls, leaves chutney, chutney powder, and ragi addai with LPC were closest to the controls. The general conclusions based on this and other studies were that: (a) The color is most acceptable in preparations which are normally green or dark in color; (b) texture is not so much a problem as LPC combines uniformly with the other ingredients; (c) the grassy flavor is a problem in preparations which do not have strong spices; and (d) the marked bitter or sour taste is a problem. It was found that strong spices or fully ripe banana flavors were most effective in masking the LPC flavor.

In earlier work Waterlow found that LPC was well tolerated and consumed by infants when used as a partial substitute for milk.

The other report at the Coimbatore meeting on acceptance of foods containing LPC was presented by O. L. Oke of the University of IFE, Nigeria. He told of adding LPC to three local dishes: (a) Oyo soup (a vegetable stew) (5 percent LPC dry basis), (b) yam pudding (16 percent LPC), and (c) maize gruel (38 percent LPC). The oyo soup is a green vegetable. The yam pudding is normally very dark in color so the LPC does not show much. The maize gruel is the normal postweaning diet in Nigeria. The native doctors usually provide all sorts of concoctions for adding to the gruel to provide strength and good health. Hence, LPC was not much of a shocker to the consumers. Trials were conducted among various classes of people, including University lecturers and professors, school children, workers, and villagers. "The supplemented foods were perfectly acceptable to everybody except for some university dons who objected to green spices on their rice. Even then most did not mind as long as the supplementation was not too high."

So much for what goes on around the world in LPC. I should like now to summarize two areas I have discussed.

All of the various processes being used to prepare LPC (leaf protein concentrate) are based on grinding and pressing the leaf material with or without ammonia addition to separate a green juice from a pressed cake. The latter in all cases is destined to become animal feed. The green juice is heated to coagulate the protein which may be treated in any of several ways depending on its intended use. If the green protein is to be used as a food, it should be washed to remove soluble solids prior to drying. This product still retains strong flavors and is green in color, but acceptance can be gained at least in special circumstances. We feel that a bland-flavored, light-colored product would gain better acceptance in the United States, and hence are directing our efforts in this direction. Research on LPC is being carried out in many countries of the world. It seems most likely that some form of LPC will become a basic food ingredient sometime in the future.

JUICE EXPRESSION FROM ALFALFA

B. E. Knuckles, M. E. Lazar, E. M. Bickoff, and G. O. Kohler
Western Marketing and Nutrition Research Division, Agricultural
Research Service, USDA, Berkeley, Calif.

Juice expressed from alfalfa contains carotene, xanthophyll, and protein. These compounds are important in the PRO-XAN process. They are contained in different parts of the plant cells. Chloroplasts contain the carotene, xanthophyll, the green pigment, chlorophyll, and some of the protein. The remainder of the protein is in the colorless jellylike cytoplasm. In order to get the chloroplasts from the cells it is necessary to rupture the tough cellulosic cell walls. For this reason, yields of carotene, xanthophyll, and protein are limited to the number of cells ruptured.

Maximum rupture of cells is obtained in the laboratory using some type of blender. Pilot-scale machines vary in efficiency of cell rupture. Some of these machines in decreasing order of cell rupture are: the "impulse render" of Chayen, hammer mills, moving knives and rolls. Except for the impulse render (5), these machines were discussed in a review of extraction and utilization of leaf protein (16).

Hammer mills pulp the material between beaters on a revolving shaft and the outer casting of the mill. The impulse render is a modified hammer mill in which excess water is added along with the material. Moving knives pulp the material in the narrow space between concentric sets of knife blades--one fixed, the other revolving at high speed.

Another consideration in the extraction of xanthophyll and protein is their separation from the fiber mass. The separation is accomplished by pressing the juice from the mass of ruptured cells. Thick mats of pulp filter out the chloroplasts thus reducing yields of protein and xanthophyll. Therefore, the thickness of the mat should be 6 millimeters or less as recommended by Davys and Pirie (8). In the laboratory small manually operated presses may be used. Figure 1 shows sketches of several presses for large-scale work.

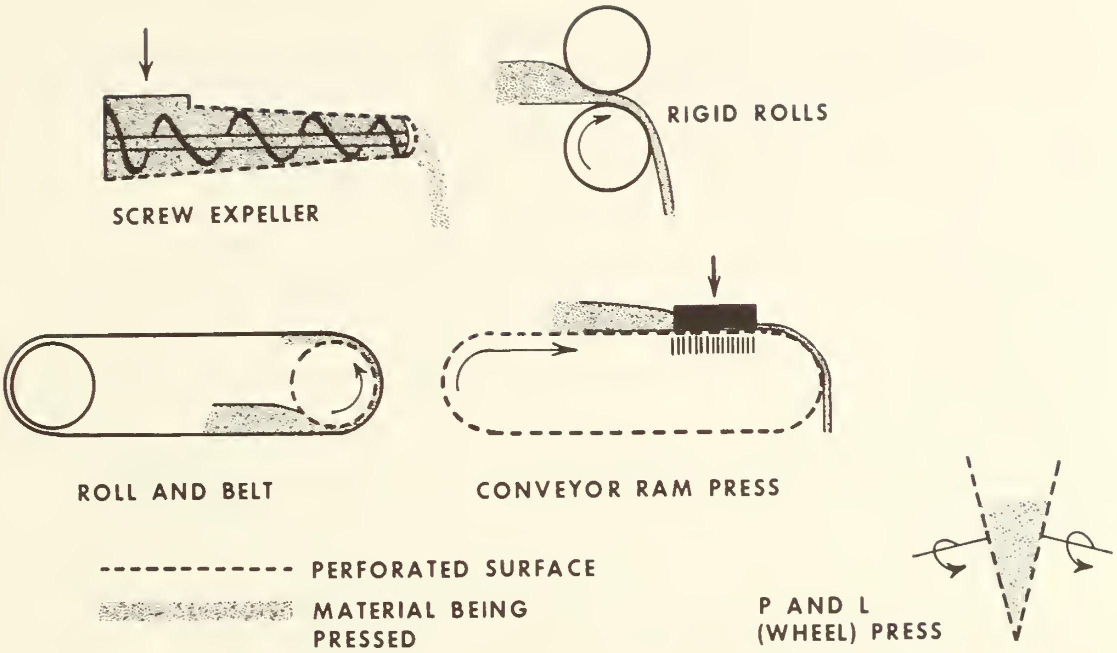


Figure 1.--Techniques used to separate liquid from fibrous pulps.

Screw expellers press the pulped material by forcing it into and through a perforated chamber by one or more screws. Rigid rolls press the pulp between fixed rolls while the combination rolls and belt arrangement presses the pulp between the belt and roll. Pirie uses a system similar to this. In a ram-type press, the material is forced against a perforated surface by a piston. Pirie is presently using the roll and belt combination (14) although he had used ram presses earlier (13). The P & L or V type press consists of two perforated disks set face to face on a shaft at an angle to one another. Material is fed in the largest space, carried around and through the narrow space where it is pressed and it is then discharged as the space enlarges again.

The selection of machines to express juice depends upon the concept or desired products of the process. Pirie and others, investigating leaf protein preparation, seek maximum extraction of protein. They consider the protein concentrate, which also contains carotene and xanthophyll, to be the major product. To achieve maximum extraction they use hammer mill-press combinations. However, Pirie is willing to sacrifice maximum extraction for simplicity (14). Simplicity is a necessity if leaf protein is to be prepared in small villages in underdeveloped areas of the world. To this end, Davys and Pirie (7) built a "Village Unit" in which 200 to 400 pounds of plant material could be extracted at a time. This unit pulps the material by a heavy roller driven around on a horizontal bed. The juice passes through perforations in the bed.

Our concept of leaf protein preparation is to have a primary product, dehydrated alfalfa, support the production of leaf protein (9, 15). To do this we extract only a portion of the protein, leaving a pressed alfalfa which can be sold as standard protein grades of dehy. The extracted protein, upon precipitation with the carotene and xanthophyll is sold as a poultry feed supplement. Emphasis is now being directed to the production of protein for human use. As the demand for human-grade protein increases, greater extraction from the plant may be desirable.

Hammer mills and moving knives were not selected for use in our PRO-XAN process for three reasons: (1) They require an additional machine to express the juice. (2) They produce a pressed cake consisting of short fiber. This short-fibered product would not be as good for dairy cattle since it would not supply long-fiber roughage. (3) These machines in conjunction with a press produce a pressed alfalfa too low in protein for sale as standard grades of dehy.

Screw expellers, P & L or V type presses, and roll mills can be used to express the juice without requiring maceration over and above that obtained in normal harvest for dehydration. They have the advantage over hammer mill-press combinations since the fiber remains long and only one machine is required to express juice.

Expellers have the disadvantage of generating excessive heat. This heat would cause the protein to precipitate and remain with the fiber. This would not matter if we wanted to remove liquid without removing the carotene, xanthophyll, and protein. Since sugar cane rolls have low power requirements and are commercially available in laboratory and commercial sizes, they were selected for use in our process. Another reason for their selection was that enough protein is left in the residue to permit the preparation of a good-quality dehy.

Rolls are now being used at a commercial plant in Brawley, Calif., where a protein-xanthophyll concentrate is being made. Each of the rolls in the cluster of three is 2-1/2 feet in diameter and 5 feet long. They can handle over 20 tons of alfalfa per hour, yielding in excess of 2,000 gallons of juice. The test rolls that we used in the development and evaluation of the rolling operation have about 1/30 the capacity of the commercial unit. Their throughput is 100 to 1,000 pounds per hour, depending upon the gap between rolls and the characteristics of the material.

Since increasing the roll gaps increases throughput but decreases juice yield, experiments were conducted to determine a good compromise between juice yield and throughput. We have found that an inlet gap of 0.4 inch, and an exit gap of 0.08 inch gave satisfactory results on alfalfa of 80 to 85 percent moisture. At these gap settings, 900 pounds of alfalfa was processed in an hour yielding 300 to 450 pounds juice. On an average, the juice yield was 360 pounds, or 40 percent of the fresh weight.

Casselman and others (4) at the Florida Experiment Station reported that screw expellers express varying amounts of juice, depending on moisture of the original material. We have also found that juice yields under a given set of conditions vary with the moisture of the alfalfa. As shown in following tabulation, high-moisture alfalfa yields more juice than low-moisture alfalfa.

Moisture content	Juice yield
Percent	Percent of fresh weight
86	50
81	40
75	36

The data given in table 1 show the order of magnitude, not actual values since the starting materials were different. We have found that the juice and protein yields from sugar cane rolls and the P & L type press are similar to yields reported for Vincent screw expellers (4) but lower than the yields from the impulse render (5) or hammer mill-press combination (6). The higher yield of the latter two machines probably resulted from the greater maceration and cell rupture received prior to expression of juice.

Table 1.--Comparison of several types of juice expressors

Investigator	Machine	Juice yield[1] 1st pass	Crude protein yield[2] 1st pass	Crude protein yield[2] 2nd pass[3]
		Percent	Percent	Percent
Knuckles	Sugar cane rolls	40	27	10
	P & L press	38	24	---
Casselman	Vincent screw expeller	40	25	---
McDonald	Powling screw expeller	50	30	15
Pirie	Hammer mill-press combination	75	50	25
Chayen	Impulse render	---	75	---

[1] Expressed as percent of fresh weight.

[2] Expressed as percent of total crude protein (N x 6.25) in fresh alfalfa.

[3] Water added prior to second pressing.

The present practice of Pirie and coworkers is to make two extractions, with water being added prior to the second. The second pressing removes one-half of the remaining protein, or about 25 percent of the total crude protein (protein being N x 6.25) (12). Thus, the protein extraction can be increased by multiple expression. Table 1 shows that expellers (11) and sugar cane rolls also remove additional protein by a second pressing.

Table 2 gives the crude protein, fiber, and ash analysis of fractions obtained from one pass through sugar cane rolls. The protein is still high in the pressed cake, making it possible to market a high-quality dehy. The ash content is low because water soluble salts and dirt are removed with the juice. This is indicated by the high ash content of the juice.

Table 2.--Analysis[1] of alfalfa, expressed juice, and pressed cake

Product	Crude protein[2]	Fiber	Ash
	Percent	Percent	Percent
Whole alfalfa - - - -	23	23	10
Pressed cake - - - -	20	28	8
Expressed juice - - -	32	<1	17

[1] Dry-weight basis.

[2] Crude protein = (N x 6.25).

Note the low fiber and high protein content of the juice. This is important since further processing by heat yields a low fiber, protein-xanthophyll concentrate for poultry feed supplements.

As you know the yellow color is an important factor in the saleability of poultry products. Therefore, we had to consider loss of xanthophylls since they are responsible for pigmentation. Carotene was also considered since it is a precursor to vitamin A.

The carotene and xanthophyll content of the fractions prepared in our pilot plant is shown in table 3. The total carotene and xanthophylls in the juice and pressed cake did not equal the amount in the original material. These figures show a loss of 3 percent for carotene and 10 percent for xanthophyll. These losses are considerably less than losses in dehydration (10). This loss in rolling is due to enzymic and oxidative reactions (1,2,3). Depending upon processing conditions, the loss can be as much as 20 percent. We can reduce the loss by

Table 3.--Carotenoid distribution upon rolling

Product	Carotene		Xanthophyll	
	Mg./lb.	Pct. of total	Mg./lb.	Pct. of total
Whole alfalfa - - -	160	100	365	100
Pressed cake - - -	150	85	350	80
Green juice - - - -	130	12	307	10

adding ammonia prior to rolling. The ammonia also enhances the green color which is another factor in the saleability of the concentrate as a poultry feed supplement.

In our studies, Santoquin, an antioxidant, did not significantly reduce carotenoid loss. Therefore, it can be assumed that the majority of the loss in rolling is due to enzymic destruction.

Summary. Of the several machines for expressing juice from plants, sugar cane rolls were selected and are presently being used in a commercial plant. They are successful because the pressed alfalfa is, upon dehydration, a high protein grade dehy. This pressed material can be passed through the dehydrator at a feed rate nearly 40 percent greater than that of unrolled alfalfa. The dehydrated pressed alfalfa also supports the processing costs for the protein-xanthophyll concentrate. The expressed juice is heated to precipitate a low fiber protein-xanthophyll concentrate for poultry feeds.

When the demand for leaf protein increases, maximum extraction and yield of protein may be desired. In this case it may be necessary to use equipment other than rolls when capital cost and throughput are considered.

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COAGULATION OF LEAF PROTEIN CONCENTRATE

Melvin E. Lazar
Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA, Berkeley, Calif.

Two major assets of dried alfalfa as a feedstuff are the protein and xanthophyll (Hunter, 1969). Normally, 15-17 percent protein is required in feed grade dehydrated alfalfa, but protein contents in alfalfa grown in some areas may be as high as 21 percent or more. Marketing the surplus nutritive values for feeding monogastric animals appears to be well worthwhile, without significantly affecting the residual feed value for ruminants.

Procedures have been studied at this laboratory for preparing a protein-xanthophyll concentrate (PRO-XAN) for use as a poultry feed supplement. The basic process consists of several operations: (1) Treating freshly harvested chopped alfalfa with gaseous ammonia; (2) pressing part of the juice (green juice) from the plant; (3) heating this juice by direct injection of steam to coagulate protein and coprecipitate xanthophyll; (4) separating the coagulum from the hot residual juice fraction (brown juice); (5) drying the coagulum to 10 percent moisture (PRO-XAN); and (6) concentrating the brown juice to 50 percent solids for use separately as a "molasses" feed supplement.

Separation of protein coagulum from brown juice after coagulation is a difficult operation. Variations in chemical and physical properties of alfalfa cause variations in the nature of coagulum obtained under constant conditions. Freshly precipitated coagulum may sink, float, or remain randomly suspended in the brown juice; it may be grainy and easy to handle or slimy and hard to dewater; it may coalesce into curds or remain fine. The separation process must be designed to handle all types with little or no change in operating procedure and with minimum labor and capital investment.

Conventional drag classifiers (Perry et al. (3)) are not suitable because of the unpredictable characteristics of the coagulum. The objective of this work was to design and operate pilot-plant equipment suitable for continuous separation of all types of coagulum from residual brown juice and to predict how the methods could be scaled up to commercial size.

Experimental

The juice extraction rolls and steam injection coagulating system have been previously described (Knuckles et al. (2), Spencer et al. (4), Witt et al. (5)). Ammonia treatment was maintained constant in all tests to obtain the optimum pH of 8.5 in the green juice. Juice was processed within 1 to 3 hours after pressing. A variable-speed positive delivery pump fed green juice to the coagulation system and reduced back-pressure fluctuations. The feed pump delivered 3/4 to 2 g.p.m. to the coagulator. Coagulator temperature was automatically controlled at 195°F. in all tests and provided complete coagulation for our tests.

It was shown in preliminary bench tests that ammoniated green juice heated to 195°F. needs 1/2 to 4 minutes hold time for coagulation and agglomeration of protein-xanthophyll fraction, plus additional time for separation from the brown juice. A 3-gallon vessel was provided after the steam injection heater to give about 2 to 3-1/2 minutes hold time for the through-put used. This and additional time in the drag separator were more than adequate to obtain good separation in all tests.

The equipment was operated continuously for periods of 2-1/2 to 4-1/2 hours in each test (except test #5). Flow rates, yields, and samples were taken only after steady state was obtained, usually after 1 hour of operation.

Pilot-Plant Equipment and Operation

Drag Separator.--A troughlike tank with sloping bottom, perforated sheet metal sidewalls, and a drag conveyor with perforated metal flights was designed and constructed (fig. 1). Brown liquor strained through the false walls into side gutters and then to the settling tank. Coagulum was dragged up the inclined discharge ramp allowing brown juice to drain back. The trough had a capacity of 3 to 4-1/2 gallons, depending on the setting of the level-control overflow tube. This volume provided 2 to 4-1/2 minutes hold time with the flows used. The false walls and the drag flights were fabricated of 20-gauge perforated stainless steel (3/32-inch diameter perforations on 5/32-inch centers, 32 percent open space). The flow velocity through the perforations in the false walls was only 3/16 inch per second at 1 g.p.m. flow. The settling rate (or floating rate) of the bulk of the coagulum must be greater than the horizontal velocity of brown juice, to escape being drawn into

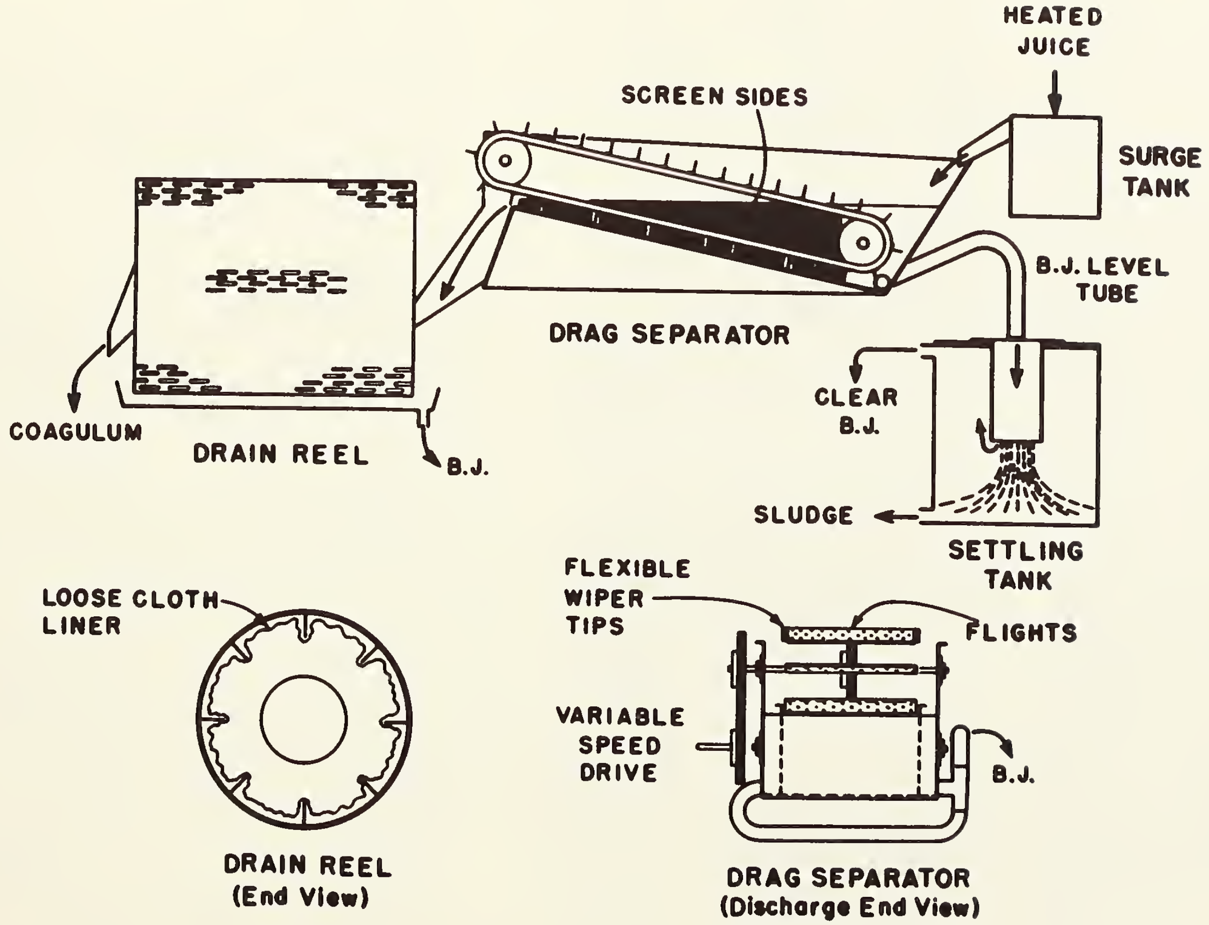

Figure 1.--Pilot plant for separation of coagulum from brown juice in the PRO-XAN process.

the screen openings. It is obviously impossible to prevent some of the coagulum from depositing on the screen or from passing through the false walls along with the brown juice. To prevent clogging of the screen, flexible silicone rubber blades were provided at each end of three of the drag flights, equally spaced, to continuously wipe the screens and remove the mat of coagulum which tended to form.

The flights of the drag separator were cleaned continuously on their return path by a curtain of fine jets of compressed air.

Drain Reel.--A slowly rotating (1 r.p.m.) perforated drum, 18 inches in diameter by 28 inches long, lined with a loose-fitting coarse cotton burlap-weave cloth (see fig. 1) was provided after the drag separator to drain the remaining free-running brown juice from the coagulum. The drum was pitched 1 inch per foot to provide a gentle slope for the tumbling coagulum to move through the unit and discharge into the collector.

Settling Tank.--A 25-gallon settling tank was provided to remove sediment that escaped the drag separator. This tank (see fig. 1), 19 inches in diameter and 22 inches deep, had a sleeve 7 inches in diameter and 9 inches long to direct the incoming brown juice down into the center of the body of liquid. An overflow pipe near the upper rim continuously drained off the clarified brown juice. At the end of the run the accumulated sediment was drained and measured.

Discussion of Results

The results of seven test runs are summarized in table 1. All runs were 2-1/2 to 4-1/2 hours long except run 5. Data were taken after steady state was achieved; results shown are averages. Data for yield of wet coagulum (19 percent solids) are shown in the last column, averaging 2.39 pounds per pound of raw-juice solids. Using these factors, and assuming a specific heat of 1.00 for juice, equations may be established for calculation of coagulum and brown-juice yields:

$$C = 0.454 \frac{FS_F}{S_c}$$

and

$$B = F\left[1 + \frac{t_c - t_F}{970} - 0.454 \frac{S_F}{S_c}\right]$$

Table 1.--Summary of performance of pilot-plant units

Run		Green juice		Coagulum			Brown juice			Coagulum yield wet[2]
				Drag separator[1]	Reel		Reel	Settling tank		
No.	Hours	Pounds/minute	Percent T.S.	Percent T.S.	Pounds/minute	Percent T.S.	Pounds/minute	Pounds/minute	Percent T.S.	
1	2-1/2	7.14	7.75	13.7	1.07	20.3	0.71	6.29	4.69	2.06
2	4-1/2	7.14	7.89	15.9	1.33	19.7	.90	4.62	4.40	2.45
3	3	7.82	8.10	13.1	1.83	18.2	1.23	5.14	4.74	2.76
4	3	7.80	8.90	16.8	1.73	18.0	.54	5.50	5.70	2.36
5	1-1/4	12.50	9.07	11.4	2.73	18.4	3.07	7.31	5.71	2.32
6	2-1/2	9.90	7.70	14.7	1.88	18.1	1.08	7.79	5.00	2.35
7	2-1/2	9.90	9.80	16.6	2.21	20.0	.72	8.20	6.20	2.40
									Average	2.39

---

[1] Average of spot samples.

[2] Pounds of coagulum (19 percent T.S.) per pound of green juice solids. (To obtain pounds of coagulum (10 percent moisture) per pound of green-juice solids, multiply values in this column by 0.21.)

where F = Feed rate of green juice, pounds/minute.
S_F = Solids content in feed juice, percent.
S_c = Solids content in coagulum, percent.
C = Coagulum, pounds/minute at S_c.
B = Brown juice, pounds/minute, total from settling tank plus reel.
t_c = Coagulation temperature, °F.
t_F = Green-juice temperature, °F.

Deviations of actual and calculated values are shown in table 2.

Table 2.--Comparison of calcualted and actual values of brown juice and coagulum

Run No.	Brown juice, pounds/minute			Coagulum, pounds/minute		
	Calculated	Actual	Percent[1]	Calculated	Actual	Percent[1]
1 - -	6.83	7.00	102	1.24	1.07	86
2 - -	6.76	5.52	82	1.30	1.33	102
3 - -	7.25	6.37	88	1.58	1.83	116
4 - -	7.06	6.04	86	1.75	1.73	99
5 - -	11.30	10.38	92	2.86	2.73	95
6 - -	9.27	8.87	96	1.91	1.88	98
7 - -	8.98	8.92	99	2.20	2.21	100

[1] Percent of calculated value.

Brown-juice yield averaged about 92 percent of the calculated value, probably because of evaporative losses from hot open system.

Capacity of the pilot plant varied with the green-juice characteristics, but our tests showed that up to 10 to 12-1/2 pounds/minute of green juice could be easily handled. One test (not shown) at 17 pounds/minute feed rate indicated that the equipment soon became overloaded.

Operation of the drag in the separator was optimum at 105 second/cycle. Higher speeds reduced coagulum drainage time, and slower speeds also reduced drainage by accumulating too much coagulum on the drag flights.

A small amount of fine coagulum escaped through the sidewall screens with the brown juice, but it was only 1.6 to 6.5 percent of the total coagulum (see table 3) and was easily removed in the settling tank. When a finer screen (0.045-inch openings) with the same open area was tested, clogging was prohibitive.

Table 3.--Performance of settling tank

Run No.	Green-juice rate	Coagulum from reel	Sediment in brown juice	Sediment, percent of coagulum[2]
	Pounds/minute	Pounds/minute[1]	Pounds/minute[1]	
2	7.14	0.262	0.017	6.5
3	7.82	.334	.021	6.3
4	7.80	.312	.005	1.6

---

[1] Dry basis.

[2] If sediment were not removed from the brown juice, it would amount to about 4 percent of the brown-juice solids.

The sediment passing through the screens with the brown juice was shown in bench tests to settle rapidly, in only 2 to 3 minutes. The settling tank provided perhaps 5 times the retention time needed but it was deliberately oversized to accumulate sediment for an entire day's run. It is common practice to continuously pump off an underflow from commercial settling tanks. In our plant the sludge could be returned to the drain reel.

At steady state in the tests the drain reel held different amounts of coagulum and as a result retention time varied (table 4).

Table 4.--Performance of drain reel

Run No.	Coagulum yield	Reel holdup at steady state[1]	Calculated retention time
	Pounds/hour[1]	Pounds	Minutes
2	80	40	30
4	104	30	18
7	133	48	22

---

[1] As-is basis.

This was attributed to different drainage characteristics of the coagulum produced on different days because of variations in alfalfa. Retention time was self-adjusting unless drain reel capacity is exceeded. The cloth liner did not clog if no more than one-third of the coagulum discharged from the drag separator was free-running brown juice (about 1/8 g.p.m.). Heavier loads of brown juice caused by coagulum characteristics or by overloading the drag separator resulted in incomplete drainage, and wetter coagulum was discharged from the reel.

Scale-Up of Equipment

To scale-up pilot-plant equipment to commercial size, only the drag separator and the drain reel are considered because scale-up of other units may be treated conventionally.

The pilot-plant drag separator has a capacity of about 3-1/2 gallons between screens when the liquid is 5 inches deep at the deep end. At 1 gallon per minute, retention time was 3-1/2 minutes with the overflow control tube set for 5-inch depth. To scale-up to 50 g.p.m., for example, volume must be 175 gallons to obtain the same retention time. Scaling up the dimensions of the pilot-plant unit by a factor of $(50)^{1/3}$, or 3.68, would give the required volume, retention time, and liquid flow rates through the screen. Similarly, to scale up to 100 g.p.m., the factor would be $(100)^{1/3}$, or 4.64. Screen openings must be retained close in size to those in the pilot-plant model, and liquid through-flow velocity must not be exceeded.

To scale-up the drain reel, the same factors should be used. Also, it may be that the ratio of cloth drain area to volume of coagulum is important, a factor which is still to be investigated.

Summary

Pilot-plant equipment was developed to separate coagulum from residual liquor after heat-precipitation of a protein-xanthophyll concentrate from alfalfa juice. In continuous tests lasting 2-1/4 to 4 hours, an average of 2.4 pounds of wet coagulum (at 19 percent solids) was produced per pound of raw green juice solids, equivalent to a yield of 0.45 pounds of PRO-XAN (10 percent moisture). The coagulum (1.6 to 6.5 percent of the total) that escaped the drag separator into the residual liquor was easily recovered in a settling tank. Scale-up 50-fold would require units with dimensions 3.68 times as large as the pilot-plant units; for 100-fold scale-up the factor would be 4.64.

Acknowledgment

The authors are indebted to R. P. Graham and J. M. Graves for the design and construction of the drain reel and to R. E. Miller for assistance with sampling and analyses.

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DEWATERING AND DRYING OF PROTEIN CURD

R. E. Miller, E. M. Bickoff and G. O. Kohler
Western Marketing and Nutrition Research Division, Agricultural
Research Service, USDA, Berkeley, Calif.

The objective of this work was to find an inexpensive, commercially feasible method of preserving protein-xanthophyll concentrate. Others have been concerned with a similar problem when making leaf protein concentrate, a product like ours but directed toward human consumption. Pickling, salting, canning, freezing, solvent extraction, and drying have all been tried with various degrees of success. Since PRO-XAN was to be used primarily as a poultry feed supplement, the problem became one of choosing a method of drying the concentrate.

Duckworth and Woodham (2) compared the utilization of leaf protein dried on a drum drier, in an oven, and freeze-dried using chickens and rats. They report the nutritive value fell seriously if the temperature of the leaf protein concentrate reached 180° F. or higher.

Morrison and Pirie (4) found that the nutritional loss can be avoided by drying in a current of air at low temperature, but the protein becomes hard and granular.

D. B. Arkcoll (1) described an air-drying process that produced a product similar to that obtained by freeze drying. The nutritive value, estimated by in vitro papain digestion, remained at a high level when care was taken to assure that the product temperature did not exceed 180° F. and the product moisture content was around 9 percent.

An alternate procedure of preparing leaf protein concentrate proposed by Hartman et al. (3) consists of spray drying the whole juice. This gives a water-soluble product with high nutritive value as estimated by the pepsin pancreatin digest index. We believe that this process would tend to concentrate water-soluble saponins, limiting the amount of leaf protein concentrate that could be fed some species of nonruminants, specifically poultry.

In all of the work just mentioned, the primary concern was the preservation of the biological value of the protein. The carotenoids were generally ignored, being considered of minor importance when compared with the protein. The emphasis of our process is placed on obtaining a high-xanthophyll, low-fiber product for broiler pigmentation.

Of the four drying methods discussed, freeze drying, the method we use most frequently for drying laboratory samples, was considered too expensive for our product. We considered spray drying but decided that it would be impractical for the size operation we had foreseen.

Both air drying and drum drying seemed promising and the availability of a pilot-model double-drum drier encouraged us to try this method first.

Drum drier.--The drum drier consisted of two 12-inch-diameter by 18-inch-long chromeplated rolls which were steam heated. The drum rotation speed was adjustable, as was the gap between the rolls.

The drum-dried product was dark green and flaky as it came from the drums and was easily powdered. Occasionally it burned and stuck to the rolls making it necessary to stop and clean the drier, however, a satisfactory product was obtained most of the time.

Drum drying.--Carotene and xanthophyll analysis of the drum-dried products demonstrated that losses could be kept below 10 percent for carotene and 15 percent for xanthophyll (table 1).

Table 1.--Carotene-xanthophyll losses during drum drying[1]

Temperature, °F.	Drum speed	Moisture	Loss	
			Carotene	Xanthophyll
	Sec./rev.	Percent	Percent	Percent
280	12	26	3	14
300	12	15	8	14
320	12	6	2	14
310	13	20	8	5
310	20	15	7	5
310	40	5	1	4

[1] Gap 0.010 inch for first three values, 0.012 inch for last three values.

The translation from pilot-scale drum drying to full-scale was not achieved. We believe the failure was due partly to the quality of the alfalfa during the test period but mostly to the poor surfaces on the large rolls. The pilot drum-drier rolls were chrome plated, while the large drum-drier rolls were fine-grain cast steel which rusted and quickly became pitted. We were unable to dry the protein-xanthophyll concentrate on these drums without it burning and sticking.

Air drying.--We next examined air drying. A fluidized bed drier about 10 feet long was transported to our collaborators' plant for testing. The wet coagulum, containing about 75 percent water after draining, required up to 10 passes on this drier equal to about 12 minutes to obtain a product moisture level of 10 percent because of the low inlet temperature. The coagulum was especially difficult to handle in the early stages of drying until a solids content of 35 to 40 percent was obtained. To improve the handling characteristics during the initial phases of drying, a recycling step was added. A portion of the previously dried coagulum was ground through a screen and mixed with the wet coagulum, usually at a ratio of 2 pounds wet to 1 pound dry. This formed a crumbly, easily handled material which could then be air dried with little trouble.

Our concern over the possible carotenoid damage resulting from such an operation proved groundless as the total losses amounted to less than 5 percent for carotene and 10 percent for xanthophyll.

The scale-up of the fluidized bed drier seemed impractical, and we decided to test our pilot-model rotary air drier. This drier has the advantage of a much higher inlet air temperature (900°-1,000° F.) when compared with the fluidized bed drier (200°-210° F.), allowing greater drying capacity. It was necessary to modify the Heil drier feed system because the wet concentrate would stick in the throat of the drier and burn, eventually plugging the drier entrance. The addition of the re-blend or add-back step, in which approximately equal volumes of wet and dry coagulum are mixed, eliminated the sticking. Particle-size buildup was prevented by grinding the dried concentrate through a 1/16-inch screen.

The carotenoid losses were low as long as the drier outlet temperature remained at or below 180° F., and the product retained 8 to 10 percent moisture (table 2).

Table 2.--Carotene-xanthophyll losses during Heil drying

Inlet	Outlet	Sample moisture	Cumulative loss during drying	
			Carotene	Xanthophyll
°F.		Percent	Percent	Percent
400-500	170-180	9.8	3	6
800-900	170-180	8.5	3	7
900-1100	170-180	8.9	5	8
400-425	195	8.0	11	11
400-425	195	6.0	18	19
800-900	225	2.0	15	26

The successful air drying of the protein-xanthophyll concentrate in the pilot rotary air drier led to the installation of a single-pass rotary air drier in our collaborator's plant. This drier is now in operation and the product is equivalent to that prepared in the Heil.

Modifications and improvements.--As an alternative to the add-back system now in use, we are investigating the possibility of mechanically removing enough liquid from the coagulum, normally 15 to 20 percent solids, to raise the solids content high enough for direct single-pass rotary air drying which would require about 35 percent solids. This would increase the drier capacity and decrease the amount of time the product is exposed to the high temperature. In addition, the dried product would contain less of the soluble solids including saponins and would show a 7 to 8 percent increase in the xanthophyll level.

In order to evaluate the mechanical expression of a portion of the brown juice, a hand press was constructed. The press was calibrated to give from 7 to 26 p.s.i. on the surface of a cylinder by sliding a weight along a lever. A pressure was applied for a period of time, and the juice volume was measured as it drained from the press. In this way it was found that 20 p.s.i. for 1 to 2 minutes would remove enough liquid to yield a concentrate approximately 40 percent solids (fig. 1).

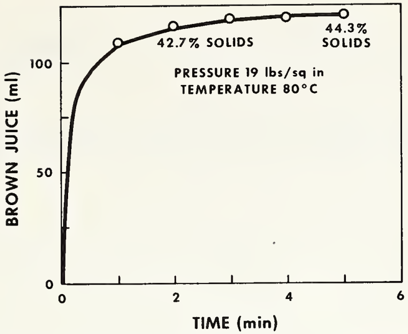

Figure 1. Juice expression from 200 g. of coagulum containing 20 percent solids by weight.

The pressed coagulum can be easily crumbled, is not sticky, and will air dry readily.

The color of the pressed and dried product is a brighter green than that of the product dried using the add-back system.

We envision a process in which the coagulum is fed directly from the coagulator onto a continuous belt-type press and from there through the dryer. We are presently evaluating two presses that are capable of doing this.

In summary, the drying of protein-xanthophyll concentrate, PRO XAN, is not an especially difficult problem. It can be done in a number of ways depending on the end use. Air drying would seem to be the best for a poultry feed supplement. A commercial dehydrating unit may conveniently be used with the addition of either the add-back or pressing and draining step. For best results, the temperature of the product should not exceed 180° F.

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YIELD AND COMPOSITION OF FRACTIONS

Donald de Fremery
Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA, Berkeley, Calif.

Although the wet fractionation of alfalfa is not a new idea, its realization as a successful commercial process has been slow in arriving. The concept of harvesting an agricultural crop, fractionating it into its component parts, and marketing the partially purified concentrates to users with specific needs appeals to the efficient businessman. Provided the economics are favorable, this technique should herald the future trend in agri-business.

The details of juice expression, of the heat-coagulation of the protein-xanthophyll complex, and of drying of the resultant protein curd have been described. I hope to fulfill two goals: (1) To present the yield and composition of the main products of the wet-fractionation system, and (2) to serve as a bridge between the earlier discussions and the two that follow. The following discussions will deal with the digestibility and quality of alfalfa protein. Consequently, my last three tables will present the amino acid composition of several alfalfa protein fractions.

Table 1 presents the yield of the various fractions of the wet-fractionation system. My yield data is for 100 pounds of fresh alfalfa whose moisture content is 80 percent. Two different levels of juice expression are presented: 35 percent (a light squeezing) and

Table 1.--Yield of various fractions of wet-fractionation system

Fraction	35 pct. juice expression		50 pct. juice expression	
	Fresh weight	Dry weight	Fresh weight	Dry weight
	Pound	Pound	Pound	Pound
Fresh alfalfa	100	20	100	20
Pressed residue	65	17.6	50	16.5
Green juice	35	2.4	50	3.5
Coagulum	6.6	1.1	9.5	1.6
Brown serum	32.9	1.3	47.0	1.9
(60 pct. serum concentrate)	(2.2)	---	(3.1)	---

50 percent (a more severe treatment). The yields of the fractions are expressed both on a fresh-weight basis, which indicates where materials handling problems might arise, and on a dry-weight basis, which is the basis for marketing the final products. On a fresh-weight basis, it is apparent that most of the product goes to the pressed residue and the brown serum, regardless of the level of juice expression. On a dry-weight basis, however, by far the largest proportion of the initial solids ends up in the pressed residue (3). These observations indicate that the wet-fractionation system is primarily a dewatering system which leaves the major portion of the product in a form which is suitable for a rapid and efficient dehydration.

The heating of the green juice and the coagulation of the protein-xanthophyll complex is accompanied by the uptake of water as steam condensate. This explains the fact that the sum of coagulum and brown serum (fresh weight) exceeds the weight of the green juice by about 13 percent. The concentration of the brown serum (about 4 percent solids) to a 60 percent solids concentrate obviously reduces the fresh weight of the serum to a marked degree.

It should be noted that the solids in the green juice are distributed about evenly between the coagulum and the brown serum.

The composition of the various fractions of the wet-fractionation system (3, 4) is shown in table 2. This is only a partial list of components, a list which includes, however, the components of greatest interest. Note that the solids are expressed as a percentage of the fresh weight, whereas, the other components are all based on dry weight. Again, two levels of juice expression are presented (35 percent and 50 percent). Assuming that one starts with alfalfa having a 20 percent solids content the solids content of the pressed residue increases to 27 percent or 33 percent, depending on the degree of juice expression. The drained coagulum, which has a consistency resembling cottage cheese, has a solids content of only 17 percent; the brown serum is a dilute fluid whose solids content is only 4 percent.

The protein and fiber concentrations change in opposite directions in the residue following juice expression. The fact that the protein concentration decreases while the fiber concentration increases reflects the fact that the green juice is removing some soluble protein and leaving the insoluble fiber behind. The protein concentration in the coagulum is more than double that in the fresh alfalfa. The coagulum is thus a high-protein, low-fiber concentrate, an excellent protein source for non-

Table 2.--Composition of various fractions of wet-fractionation system

Component	Fractions				
	Fresh alfalfa	Pressed residue		Coagulum	Serum
		35 percent juice	50 percent juice		
Solids, percent[1]	20	27	33	17	4
Protein, percent	23	21	20	55	22
Fiber, percent	23	26	27.5	3	0
Carotene, mg./lb.	140	137	134	350	0
Xanthophyll, mg./lb.	320	313	306	800	0

[1] Solids expressed as percent fresh weight; others based on dry weight.

ruminant nutrition. The value of 22 percent for protein concentration in serum is based on total nitrogen times the factor 6.25. Only about half of this nitrogen is contained in true protein or amino acids.

The pigments, carotene and xanthophyll, are decreased only slightly in the pressed residue. None of the pigments in the green juice remain in the brown serum; they are precipitated with the proteins in the coagulum. The very high levels of carotene and xanthophyll in the coagulum are theoretical values only. They assume that there are no losses during crushing, heat coagulation, and drying of the coagulum. Since these losses are quite variable, only the theoretically maximum values are presented.

The data that are in tables 1 and 2 have been rearranged and are presented in table 3. This table shows the flow of components through the wet-fractionation system with 50 percent juice expression. For example, 42 percent of the water in fresh alfalfa remains with the pressed residue, and 58 percent passes into the green juice. The interesting point about table 3 is the comparison between the distribution of water and the distribution of the solid components. Although most of the water appears in the green juice, 70 to 80 percent of the solid components remain in the pressed residue.

Table 3.--Flow of components through wet-fractionation system
[50 percent juice expression]

Fraction	Component			
	Water	Solids	Protein	Xanthophyll
	Percent	Percent	Percent	Percent
Fresh alfalfa	100	100	100	100
Pressed residue	42	82.5	72	79
Green juice	58	17.5	28	21
Coagulum	10	8	19.5	21
Brown serum	56	9.5	8.5	0

The further distribution of the solid components which appear in the green juice is interesting also. Although slightly more than half of the total solids end up in the brown serum, note the preferential retention of protein and xanthophyll in the coagulum.

The mineral composition of brown serum is presented in table 4. The data are presented on a dry-weight basis as well as on a volume basis. The latter basis assumes that the serum has been concentrated to a 60 percent solids syrup whose density is 1.32 g./ml. Potassium is the major cation present, followed by magnesium, calcium, sodium, and trace elements.

Table 4.--Mineral composition of brown serum

Mineral	Serum solids	Serum concentrate[1] (60 percent solids)
	Percent	G./liter
Potassium	8.8	70
Chloride	1.25	9.9
Magnesium	1.03	8.2
Calcium	.58	4.6
Sodium	.186	1.47
Phosphorus	.155	1.23
Zinc	.0106	.084
Iron	.0098	.078
Manganese	.0044	.035
Copper	.0007	.006

[1] Density of concentrate, 1.32 g./ml.

The amino acid composition of the coagulum and of the brown serum is presented in table 5. The wet, drained coagulum as normally prepared has a moisture content of about 83 percent (see table 2). The high moisture content, of course, represents contamination by the brown serum, all of whose solids are soluble. This contamination means that the dried unwashed coagulum has a soluble solids content of about 25 percent (as a percentage of the total solids). Pressing and/or washing the coagulum before drying will reduce the soluble solids content markedly. The values in the table represent the amino acid composition of coagula whose soluble solids content is 25, 10, or 1 percent, respectively, as well as the composition of the brown serum itself. It can be seen that washing out the soluble solids (salts, sugars, some amino acids, etc.) has the net effect of increasing the concentration of each amino acid in the coagulum. This means that the recoverable amino acids increase from 54 percent of the dry weight to 69 percent as the soluble solids content is reduced to 1 percent. It is also interesting to compare the amino acid composition of brown serum in this table with the protein com-

Table 5.--Amino acid composition of coagulum and brown serum

Amino acid	Soluble solids in coagulum			
	25 percent	10 percent	1 percent	Brown serum
	Percent	Percent	Percent	Percent
Aspartic acid	5.9	6.6	7.1	2.1
Threonine	3.0	3.5	3.8	.5
Serine	2.5	2.9	3.2	.5
Glutamic acid	6.3	7.2	7.8	1.6
Proline	2.7	3.1	3.3	.6
Glycine	3.0	3.5	3.8	.5
Alanine	3.5	4.0	4.4	.7
Cystine	.7	.8	.9	.2
Valine	3.7	4.4	4.8	.5
Methionine	1.1	1.3	1.4	.1
Isoleucine	3.0	3.6	3.9	.4
Leucine	5.1	6.0	6.6	.5
Tyrosine	2.5	2.9	3.2	.3
Phenylalanine	3.3	3.9	4.2	.4
Lysine	3.3	3.9	4.2	.5
Histidine	1.4	1.6	1.7	.2
Arginine	3.4	4.0	4.4	.3
Total	54.4	63.2	68.7	9.9

position of brown serum from table 2. As stated previously, about half of the nitrogen in brown serum is contained in nonprotein, nonamino acid compounds.

The amino acid composition of various leaf protein fractions is presented in table 6. These data have been taken from a recent paper by Byers of the Rothamsted Experimental Station (1). They represent the average amino acid composition of leaf protein fraction from three different species of plants: barley, lupin, and Chinese cabbage. These protein fractions were obtained from the expressed green juice, not from whole leaf. The "unfractionated" fraction represents the total juice protein precipitated either by heating to 80°C. or by exposure to 5 percent trichloroacetic acid. The chloroplastic fraction consisted of the proteins contained in the washed sediment from low-speed centrifugation, and the cytoplasmic fraction consisted of the proteins contained in the supernatant from high-speed centrifugation. Although careful examination reveals that there are many differences in amino acid content between the various fractions, only those differences in which the chloroplastic and cytoplasmic fractions differ from

Table 6.--Amino acid composition of leaf protein fractions[1]

Amino acid	Protein fraction		
	Unfractionated	Chloroplastic	Cytoplasmic
Aspartic acid	9.9	9.9	9.9
Threonine	5.1	4.9	5.3
Serine	4.5	5.1	4.1
Glutamic acid	11.7	11.2	12.1
Proline	4.7	5.0	4.6
Glycine	5.6	6.2	5.3
Alanine	6.3	6.6	6.1
Cystine	1.6	1.0	1.9
Valine	6.2	6.0	6.3
Methionine	2.0	2.1	2.1
Isoleucine	4.8	5.3	4.5
Leucine	9.5	10.5	8.8
Tyrosine	4.6	4.3	5.2
Phenylalanine	6.2	7.0	5.8
Lysine	6.8	5.2	7.2
Histidine	2.4	1.9	2.7
Arginine	6.6	6.3	6.9

[1] g. amino acid per 100 g. recovered amino acids.
Source: Byers (1).

each other by more than 10 percent will be mentioned here. The cytoplasmic protein is higher than the chloroplastic protein in its content of cystine, tyrosine, lysine, and histidine. Conversely, the chloroplastic protein is higher in its content of serine, glycine, isoleucine, leucine, and phenylalanine. The possible use of the cytoplasmic proteins for human feeding is bolstered by the higher content of cystine; in general, leaf proteins are deficient in the sulfur amino acids. Additionally, the higher content of lysine is also encouraging. Although lysine is in adequate supply in leaf proteins, the use of blends of leaf proteins with seed and/or cereal proteins (which are generally deficient in lysine) is made more likely by the use of the cytoplasmic fraction of leaf proteins.

The low level of isoleucine in this latter fraction, however, is discouraging for human nutrition. The level of isoleucine is low enough that it could become the limiting essential amino acid, particularly in cases of methionine supplementation. This is shown more clearly in table 7.

Table 7.--Essential amino acid pattern of selected proteins

Amino acid	Hen's egg (whole)	Soybean meal	Whole leaf protein	Chloroplastic protein	Cytoplasmic protein
	A/E value (mg. amino acid/g. total essential amino acids)				
Leucine	172	187	193	217	179
Isoleucine	129	123 (95)[1]	99 (77)	110 (85)	92 (71)
Valine	141	128 (91)	127 (90)	123 (87)	128 (91)
Threonine	99	97	104	101	107
Lysine	125	148	139	107 (86)	146
Tryptophan	31	43	43	44	43
Total aromatic amino acids	195	200	222	233	224
Total sulfur amino acids	107	74 (69)	72 (67)	65 (61)	81 (76)
Cystine	46	38 (83)	32 (70)	21 (46)	39 (85)
Methionine	61	36 (59)	40 (66)	44 (72)	42 (69)
	E/T value (g. total essential amino acids per g. total nitrogen)				
	3.22	2.44	3.53	3.55	3.52

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[1] Numbers in parentheses are A/E values as a percentage of hen's egg values.

Table 7 presents the essential amino acid pattern of hen's egg, soybean meal, and leaf protein fractions (1, 2). At this point it may be wise to define E/T value and A/E value. The E/T value is the ratio of the grams of total essential amino acids in a protein to the grams of total nitrogen in that protein. It is a measure of the proportion of a protein's amino acids which are present as essential amino acids. This number, characteristic for each protein, should not be less than 2.0, according to the Food and Agriculture Organization (2). The A/E value is defined as the ratio of the milligrams of a particular essential amino acid in a protein to the grams of total essential amino acids in that protein. Each essential amino acid in a particular protein thus has an A/E value associated with it. The A/E values can also be expressed as a percentage of the A/E values for hen's egg (a suitable reference protein), as has been done in table 7 for all values that are 95 percent or less of the hen's egg value. The A/E method of expressing essential amino acid content does not give the total level of essential amino acids within the protein, as the E/T value does, but it gives a good picture of the balance of the essential amino acids among themselves. This important factor, the pattern of the essential amino acids within the protein, is often overlooked in discussions of protein quality.

The numbers in parentheses in table 7, which are the A/E values as a percentage of the hen's egg values, also serve as an index of the limiting essential amino acid; for a given protein, the lowest percentage is associated with that amino acid which is limiting in that protein. For example, methionine is limiting both in soybean meal (59 percent) and in whole leaf protein (66 percent). Although it is interesting to note that the sulfur amino acids are generally deficient in the leaf protein fractions, it is also important to note that the isoleucine content of the cytoplasmic protein is approaching that of a limiting amino acid. This could be particularly serious in cases where methionine supplementation was employed.

In summary, I would like to emphasize just four points.

1. The wet-fractionation process removes the bulk of the water from the alfalfa, increasing the efficiency of dehydration of the pressed residue.

2. Dehy from the pressed residue is still the major product.

3. An important byproduct is PRO-XAN, a high-protein, high-xanthophyll, low-fiber product. It is ideal for poultry nutrition.

4. The protein quality of leaf protein is good, in spite of the low sulfur amino acid content. Although the mild isoleucine deficiency of the cytoplasmic protein is discouraging, the enrichment in cystine and lysine is most promising for human nutrition.

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IN VIVO AND IN VITRO PROTEIN DIGESTIBILITY OF ALFALFA AND CONCENTRATES

A. N. Booth, R. M. Saunders, M. A. Connor, and G. O. Kohler
Western Marketing and Nutrition Research Division, Agricultural Research Service, USDA, Berkeley, Calif.

The potential of plant protein is now widely recognized as a source of good-quality protein that can be made readily available in many countries where protein is deficient. Studies in England, India, and the United States indicate that alfalfa is one of the most promising crops, and numerous publications have appeared attempting to solve the inherent processing problems. In the most recent paper by Byers of Rothamstead Experimental Station, England (3), it was concluded that some loss of nutritive value occurs during processing of leaf protein, but at which point or points is not known. In her closing remarks Byers suggests that in vitro studies will be used to determine the state at which nutritive value deteriorates. This report describes in vitro evaluation studies of alfalfa protein and demonstrates the processing stages where nutritive value is lost.

This laboratory has long been interested in the effect of dehydration on the labile nutrients of alfalfa. In a presentation at the 10th Technical Alfalfa Conference, it was shown that during dehydration of alfalfa losses up to three-fourths of the xanthophyll and one-third of the tocopherols could occur. In addition, losses of carotene and amino acids are significant. If we go back in the literature, it is obvious that changes also occur in protein quality and digestibility during drying. Clandinin in 1949 and Carpenter in 1957, for example, found losses in nutritive value after moist heating of protein, and concluded this was due to actual decreases in protein digestibility. Since these early observations, many workers have made similar studies, including observations on plant protein concentrates. Buchanan (2) in 1969 showed that heating wheat leaf protein at 60° C. caused only a small decrease in protein digestibility but heating at 100° caused much larger losses. These losses were attributed to two different reactions: (1) A lipid-complexing reaction which depends on moisture content but not on the presence of oxygen and (2) modification of the protein. It should be pointed out that Buchanan measured these changes in protein digestibility with an in vitro system which is essentially a papain digestion. Unfortunately, this in vitro system showed very poor correlation with in vivo feeding trials, and for this reason we proceeded to develop our own in vitro system for the evaluation of protein characteristics during processing.

In this laboratory we are interested in manufacturing protein concentrate from alfalfa with two general objectives: (1) Preparation of a concentrate high in protein, xanthophyll, and carotene to be used in poultry rations, and (2) fractionation of the crude plant protein into cytoplasmic protein for possible human consumption and chloroplastic protein for poultry. Obviously any detrimental effects on protein digestibility and on xanthophyll content must be avoided. Our studies have thus been directed toward minimizing any harmful effects on these constituents during the processing, and thus to predict optimum processing conditions.

To facilitate this work we have used two in vitro systems to follow protein digestibility. In one system the sample is digested first with pepsin and then with trypsin. This system is similar to the pepsin-pancreatin index method of Akeson and Stahmann (1) but correlates better with in vivo data. Our second in vitro system involves digestion with pronase, a fungal protease. Correlation of the pronase system with in vivo data is not nearly as good as the pepsin-trypsin method, but the extremely simple procedure warrants its use for specifically following changes in protein digestibility. In actual fact the in vitro pronase digestibility figures are about 10 percent below the in vivo or animal digestibility values. Incidentally, the in vitro protein digestibility is calculated simply by dividing the value for nitrogen in the undigested sample into the undigested nitrogen minus the water insoluble nitrogen left after incubation.

Table 1 shows the correlation between the in vitro and in vivo

Table 1.--In vitro and in vivo (rats) protein digestibility values for alfalfa products

Source of protein	Protein digestibility	
	In vitro	In vivo
Freeze-dried coagulum 1 - - - - - - - - - - -	76.6	79.1
1, Water-washed - - - - - - - - - - - - - - - -	83.4	85.7
Freeze-dried coagulum 2 - - - - - - - - - - -	77.5	85.5
Freeze-dried coagulum 3 - - - - - - - - - - -	83.0	86.0
Commercial coagulum 1 - - - - - - - - - - - -	79.7	81.9
Commercial coagulum 2 - - - - - - - - - - - -	81.3	80.5
Leaf dehy -	--	65.2
Stem dehy -	--	62.1
Whole meal dehy - - - - - - - - - - - - - - - -	--	62.5

values of various alfalfa products. In five out of six cases the in vitro values were slightly lower. For comparative purposes the in vivo digestibility values for alfalfa leaf, stem, and whole meal are included. These lower values are presumably due to part of the protein being bound in the cellular structures and consequently unavailable to the monogastric animal.

A graphical representation (fig. 1) is seen of the correlation between in vitro and in vivo protein digestibilities which really are quite good for most practical purposes. This is the same data as shown in table 1.

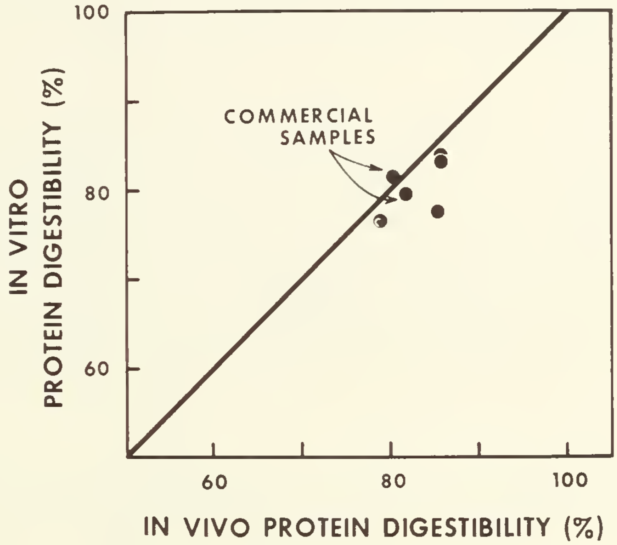

Figure 1. Correlation between in vitro and in vivo protein digestibilities.

Since additional research work is still underway, this report is far from complete, but the following figures will give you an idea of the problems arising during alfalfa processing. Figure 2 shows the instability of certain essential amino acids in alfalfa meals as the moisture content is decreased. This figure was provided by Mr. Livingston and Mrs. Allis of our Field Crops group. It is obvious that there is a decrease in amino acids, particularly lysine, as moisture content decreases, or in other words as the dryer temperature increases. As mentioned earlier, xanthophyll and carotene contents also decrease as the dryer temperature

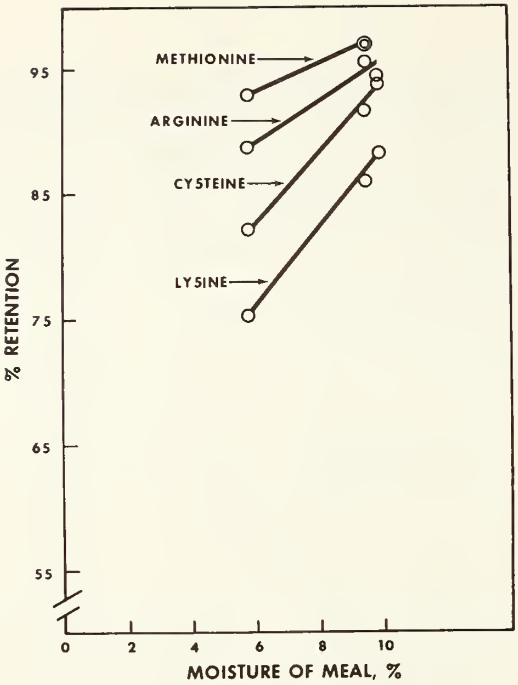

Figure 2. Effect of moisture content on the amino acids in alfalfa meals.

increases. Figure 3 summarizes some work on dehydrated alfalfa

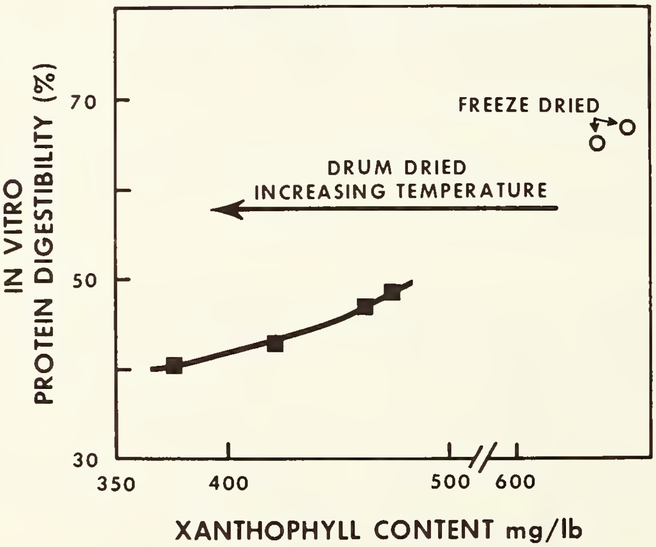

Figure 3. Effect of temperature on protein digestibility and xanthophyll content.

leaf. Protein digestibility decreased as the dryer temperature increased from 250 to 270-290° F., and the xanthophyll content decreased substantially. If we take freeze-dried leaf as an optimum control, these decreases are even more substantial. It is interesting that an almost linear relationship exists between xanthophyll content and protein digestibility. This apparent relationship has also been observed in dehydrated whole alfalfa and alfalfa protein concentrate. Though not shown here, a similar linear relationship exists between carotene content and protein digestibility in the same alfalfa products, i.e., they both decrease as the dryer temperature increases.

Since these detrimental effects occur during heating, in the present production of protein concentrate we must contend with not one, but two heating steps. In the normal process, after squeezing the alfalfa to yield the juice, the protein is coagulated by steam-injection at about 80-85° C. The coagulum is then removed, dewatered, and dried by heating.

Figure 4 shows the effects of coagulating the protein in the pressed juice with steam injection. Protein digestibility decreased rapidly during the first hour or so and gradually decreased throughout the test period. It would thus seem essential, in order to maintain good protein digestibility and presumably high xanthophyll, that the protein coagulum should be removed as soon as possible after coagulation.

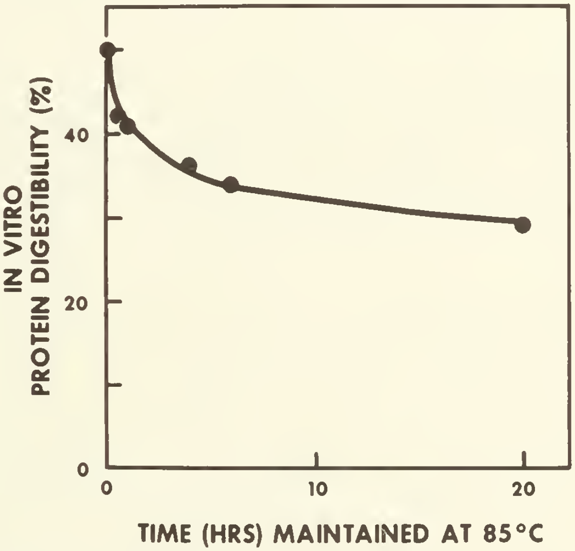

Figure 4. Effect of time at 85° C. on in vitro protein digestibility.

The second heating step in this process is of course the drying of the coagulum. Figure 5 summarizes the data. Here again, if we take the freeze-dried coagulum as the control, it has a very high xanthophyll content and reasonably good protein digestibility. In the dried product, as the drier temperature increased, both xanthophyll content and protein digestibility decreased.

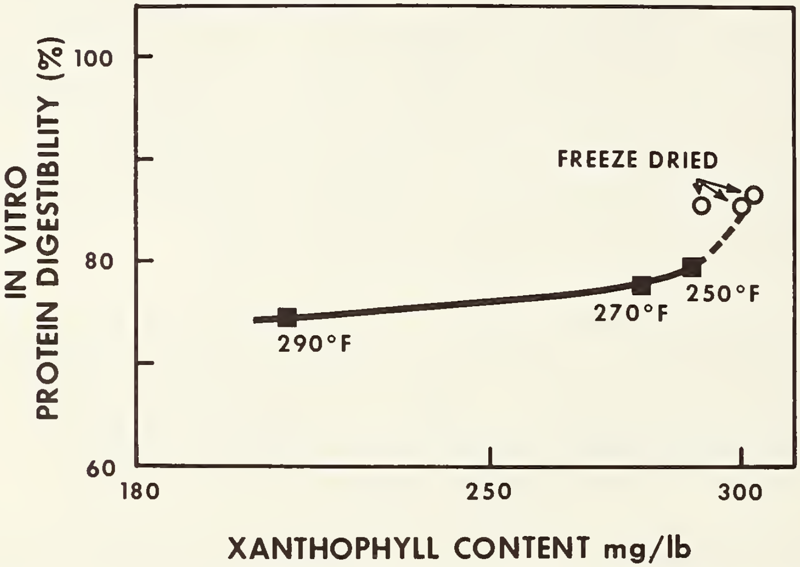

Figure 5. Effect of temperature on in vitro protein digestibility and xanthophyll content.

Knowledge of these facts, combined with the results of other workers, has prompted the research workers here at Albany to investigate other means of preparing alfalfa leaf protein whereby heating is minimal. Without going into detail, we can say that precipitation of the protein at pH's from 3.2 to 4.8, instead of heat precipitation, has produced a protein product with superior (in vitro) digestibility, in the order of 98 percent. Furthermore, careful preparation of the cytoplasmic protein (i.e. the white protein) has yielded material with 98 to 100 percent digestibility, as measured by the in vitro technique.

In spite of the problems briefly mentioned here, the overall potential for alfalfa protein concentrate is very encouraging. This is immediately apparent in table 2. Protein Efficiency Ratios, or PER's, have been measured on some of our specially prepared protein concentrates and on commercial material. As predicted from in vitro studies, the freeze-dried

Table 2.--PER values and final body weights for alfalfa protein fed to rats with and without methionine supplementation

Dietary source of protein	Percent protein	0.4 percent methionine	Final mean body weight	PER (corrected)
Casein control	10	-	143	2.50
Casein control	15	+	247	2.50
Freeze-dried coagulum 1	10	-	136.4	2.12
Freeze-dried coagulum 2	15	-	176	1.88
Freeze-dried coagulum 2	15	+	266	2.40
Commercial coagulum 2	15	-	175	1.78
Commercial coagulum 2	15	+	248	2.08

coagulum, where the second heating step has been avoided, has excellent nutritional properties, especially at the 15 percent protein level in the diet with methionine supplementation. Here the PER is comparable to that of casein and the final body weight is actually superior to casein. Even the commercial material supplemented with methionine shows a good final body weight and an acceptable PER value.

In summary, the feasibility of using a rapid in vitro system to follow protein digestibility changes during processing has been demonstrated. Together with other analytical parameters including xanthophyll and carotene levels, this should make it possible to develop the most ideal process for alfalfa protein production. Our in vivo studies with rats clearly indicate that with added methionine, alfalfa protein is equivalent to casein in nutritive value.

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UTILIZATION OF PRO-XAN AS A PROTEIN SOURCE FOR BROILERS

D. D. Kuzmicky, G. O. Kohler, and E. M. Bickoff
Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA, Berkeley, Calif.

At the present time PRO-XAN is used in poultry rations primarily as a xanthophyll or pigment source. As such with its relatively high xanthophyll content (up to about 600 mg. per lb.), levels of the order of 5 percent or less could provide all the xanthophyll needed for broiler skin and egg-yolk pigmentation.

This report concerns the investigation on the use of PRO-XAN at much larger levels to provide a primary protein source for broiler starter rations. A commercially prepared lot of PRO-XAN was used for the two chick experiments in this study.

The proximate and mineral analysis of the main ingredients used in these experiments are shown in table 1. The ether extract and ash content of this PRO-XAN are considerably higher than that of soybean meal. The ether extract if all utilized by the chick would tend to increase the metabolizable energy of PRO-XAN. However, the high ash content with the high sodium, potassium,

Table 1.--Composition of feed ingredients

Components	PRO-XAN	Soybean meal	Yellow cornmeal
		Percent	
Moisture	11.04	11.23	15.05
Protein	38.81	45.55	9.06
Ether extract	6.21	1.63	5.01
Fiber	2.36	3.93	1.24
Ash	19.2	5.48	1.80
Ca	2.35	.31	.004
Available P	.44	.29	.10
Na	.30	.04	.02
K	1.07	1.75	.33
Mg	.35	.48	.14
Cl	1.01	.08	.04

magnesium, and chloride could cause diarrhea and possibly growth depression if PRO-XAN were used at high levels in the ration. However, this high mineral content can be reduced by removing more brown juice solids or soluble solids from the PRO-XAN.

Table 2 shows the amino acid composition of the ingredients.

Table 2.--Amino acid composition of ingredients
(percent of ingredient)

Amino acid	PRO-XAN	Soybean meal	Yellow cornmeal
Lysine- - - - - - - - - - -	2.15	2.51	.25
Methionine- - - - - - - -	.79	.77	.23
Cystine - - - - - - - - - -	.43	.72	.24
Methionine + cystine- - -	1.22	1.49	.47
Arginine- - - - - - - - - -	2.19	3.06	.38
Histidine - - - - - - - - -	.88	1.06	.26
Glycine - - - - - - - - - -	2.05	1.86	.32
-Serine - - - - - - - - - -	1.76	2.33	.44
Leucine - - - - - - - - - -	3.44	3.33	1.09
Isoleucine- - - - - - - -	2.04	2.07	.32
Phenylalanine - - - - - -	2.19	2.11	.42
Tyrosine- - - - - - - - - -	1.62	1.49	.30
Phenylalanine + tyrosine-	3.81	3.60	.72
Valine- - - - - - - - - - -	2.55	2.11	.41
Threonine - - - - - - - - -	1.95	1.75	.31

Only the lysine and methionine plus cystine content of PRO-XAN is of concern here. The lysine, if all available, is adequate; however, the methionine plus cystine is a bit low.

Table 3 shows the ration compositions used in the first experiment. All the rations were calculated to contain 24 percent protein and 1440 kcal. metabolizable energy per pound. (An M.E. value of 1180 kcal./pound was estimated for the PRO-XAN.) If the alfalfa saponin was concentrated in PRO-XAN it could cause a growth depression. Therefore, cholesterol was added to the last diet since cholesterol complexes with saponin alleviating the growth depression.

The 54-percent level of PRO-XAN was intentionally made this high so that any possible deleterious effects from its use might become apparent.

Table 3.--Ration compositions, 71-035
(percent of ration)

Ingredient	Rations[1] Corn-soy	27 percent PRO-XAN	54 percent PRO-XAN	54 percent PRO-XAN + cholesterol
Yellow cornmeal- - -	43.52	37.66	31.79	31.79
Soybean meal - - - -	43.78	21.89	---	---
PRO-XAN- - - - - - - -	---	27.09	54.18	54.18
Corn oil - - - - - - -	7.00	7.00	7.00	7.00
Vitamin mix WU-24- -	1.00	1.00	1.00	1.00
Mineral mix MK-5 - -	3.00	3.00	3.00	3.00
$CaCO_3$- - - - - - - - -	1.50	.75	---	---
dl-Methionine- - - -	.20	.20	.20	.20
Solka floc - - - - - -	---	1.41	2.83	1.83
Ethoxyquin - - - - - -	.0125	.0125	.0125	.0125
Cholesterol- - - - -	---	---	---	1.00

[1] All rations are calculated to contain 24.0 percent protein and 1440 kcal. metabolizable energy per pound.

For all the rations the vitamins and amino acids were calculated to meet or exceed the NRC requirements. Methionine was added to all the rations to make sure that the methionine or methionine plus cystine content exceeded the chicks requirement. For this experiment day-old white-rock cockerels were fed a control ration for 2 weeks and then the experimental rations for 2 weeks. There were four replicates of seven chicks per replicate for each ration.

Table 4.--Chick weight gain and feed efficiency (gain/feed) data, 71-035[1]

Ration	Average gain/chick[2] Grams	Feed efficiency[2]
Corn-soy- - - - - - - - - - -	459 a	0.677 a
27 percent PRO-XAN- - - - -	458 a	.638 b
54 percent PRO-XAN- - - - -	442 a	.583 c
54 percent PRO-XAN + cholesterol - - - - - -	444 a	.600 c

[1] Day-old white-rock cockerels were fed a safflower-cornstarch ration for 14 days followed by the experimental rations for 14 days. There were 4 replicates of 7 chicks per replicate for each ration. Average chick starting weight, 201 g.

[2] Means not followed by the same letter are significantly different (P = 0.05) by Duncan's multiple range test.

Table 4 shows the chick weight gain and feed efficiency data from this experiment. The values shown are averages from the four replicates on each diet. There was no significant difference in chick weight gain from any of the rations, even though the chicks on the 54 percent PRO-XAN rations produced extremely wet excreta. Apparently, there is very little, if any, saponin in PRO-XAN since there was no significant difference in either chick weight gain or feed efficiency from the two 54 percent PRO-XAN rations(with and without cholesterol). The feed efficiency significantly decreases with increasing level of PRO-XAN and a significant linear regression is obtained. This very likely is due to either an overestimation of the metabolizable energy in PRO-XAN or the lysine in PRO-XAN being only partially available to the chick or both. The severe diarrhea was probably caused by the high mineral content of the PRO-XAN rations.

An attempt to correct the diarrhea problem was made in the next experiment by reducing the dietary PRO-XAN levels and adjusting the salt mixture. The available lysine and again saponin in PRO-XAN were also examined.

The ration compositions for this experiment are shown in table 5. As in the first experiment all the rations were calculated to contain 24.0 percent protein (without the lysine addition) and 1440 kcal. metabolizable energy per pound. The corn-soy control ration is the same, but the PRO-XAN levels have been reduced to 5, 10 and 20 percent, with lysine and cholesterol additions to two of the 20 percent PRO-XAN rations. All the PRO-XAN rations have adjusted mineral mixtures except the last one which has the same mineral mixture as the corn-soy ration and the rations in the previous experiment.

The mineral composition of the rations is shown in table 6. Of course, the NRC requirements are met and in most cases greatly exceeded. The levels of calcium, except for the last ration, available phosphorous, sodium, and magnesium are about the same for all diets. Only the potassium and chloride vary appreciably with the dietary PRO-XAN level. At these mineral concentrations of the PRO-XAN diets, it does not appear that the excreta moisture should be any worse than that from the corn-soy ration.

Table 7 shows the amino acid composition of the rations with all the NRC requirements being met or exceeded. At this point it is questionable whether all or part of the methionine addition was necessary; however, this will be a subject for a future study. The lysine content of the unsupplemented PRO-XAN rations is right on the requirement. If this lysine is not all available, this should be reflected in the chick response from the lysine supplemented rations.

Table 5.--Ration compositions,[1] 71-154 (percent of ration)

Ingredient	Corn-soy	5 percent PRO-XAN	10 percent PRO-XAN	20 percent PRO-XAN	20 percent PRO-XAN + lysine	20 percent PRO-XAN + lysine + cholesterol	20 percent PRO-XAN + K + Mg
	Rations						
Yellow cornmeal	43.52	42.44	41.36	39.19	39.19	39.19	39.19
Soybean meal	43.78	39.74	35.70	27.62	27.62	27.62	27.62
PRO-XAN	---	5.00	10.00	20.00	20.00	20.00	20.00
Corn oil	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Vitamin mix WU-24K	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix MK-5	3.00	.75	1.50	---	---	---	3.00
Mineral mix MK-6	---	2.25	1.50	3.00	3.00	3.00	---
$CaCO_3$	1.50	1.13	.75	---	---	---	.95
dl-Methionine	.20	.20	.20	.20	.20	.20	.20
l-Lysine·HCl	---	---	---	---	.25	.25	---
Solka floc	---	.50	1.00	1.99	1.74	1.24	1.04
Ethoxyquin	.0125	.0125	.0125	.0125	.0125	.0125	.0125
Cholesterol	---	---	---	---	---	.50	---

[1] All rations are calculated to contain 24.0 percent protein without lysine addition and 1440 kcal. metabolizable energy per pound.

Table 6.--Mineral composition of rations, 71-154 (percent of ration)

Mineral	Corn-soy	5 percent PRO-XAN	10 percent PRO-XAN	20 percent PRO-XAN	20 percent PRO-XAN + lysine	20 percent PRO-XAN + lysine + cholesterol	20 percent PRO-XAN + K + Mg	NRC requirement
	Rations							
Calcium	1.25	1.24	1.23	1.20	1.20	1.20	1.45	1.0
Available phosphorus	.50	.51	.52	.54	.54	.54	.51	.50
Sodium	.28	.29	.31	.33	.33	.33	.33	.15
Potassium	1.11	1.04	.97	.83	.83	.83	1.03	.2
Magnesium	.30	.29	.28	.26	.26	.26	.28	.05
Chloride	.26	.31	.36	.45	.45	.45	.45	?

Table 7.--Amino acid composition of rations, 71-154 (percent of ration)

Amino acid	Rations							
	Corn-soy	5 percent PRO-XAN	10 percent PRO-XAN	20 percent PRO-XAN	20 percent PRO-XAN + lysine	20 percent PRO-XAN + lysine + cholesterol	20 percent PRO-XAN + K + Mg	NRC requirement
Lysine	1.25	1.25	1.25	1.25	1.45	1.45	1.25	1.24
Methionine	.64	.64	.65	.66	.66	.66	.66	.46
Cystine	.42	.41	.40	.38	.38	.38	.38	.40
Methionine + cystine	1.06	1.05	1.05	1.04	1.04	1.04	1.04	.86
Arginine	1.50	1.48	1.46	1.43	1.43	1.43	1.43	1.39
Histidine	.58	.58	.57	.57	.57	.57	.57	.46
Glycine	.95	.98	1.00	1.05	1.05	1.05	1.05	1.14[1]
Serine	1.21	1.20	1.19	1.17	1.17	1.17	1.17	--
Leucine	1.93	1.96	1.98	2.03	2.03	2.03	2.03	1.59
Isoleucine	1.05	1.06	1.08	1.11	1.11	1.11	1.11	.85
Phenylalanine	1.11	1.13	1.15	1.19	1.19	1.19	1.19	.79
Tyrosine	.78	.80	.82	.85	.85	.85	.85	.69
Phenylalanine + tyrosine	1.89	1.93	1.97	2.04	2.04	2.04	2.04	1.48
Valine	1.10	1.14	1.18	1.25	1.25	1.25	1.25	.99
Threonine	.90	.92	.95	1.00	1.00	1.00	1.00	.79

[1] Requirement can be supplied by glycine and/or serine.

For this experiment day-old white-rock cockerels were fed a control ration for 7 days followed by the experimental rations for 19 days. As in the previous experiment there were four replicates of seven chicks per replicate for each ration.

The chick weight gain and feed efficiency data are shown in table 8. Analysis of the data indicates that there was no significant difference in chick weight gain from any of the rations. Consequently, there was no detectable effect on chick growth from dietary PRO-XAN levels, lysine and cholesterol additions, and variation in ration mineral content.

Table 8.--Chick weight gain and feed efficiency (gain/feed) data, 71-154[1]

Ration	Average gain chick[2]	Feed efficiency[2]
	Grams	
Corn-soy - - - - - - - - - - - - - - -	578 a	0.701 a
5 percent PRO-XAN - - - - - - - - -	577 a	.701 a
10 percent PRO-XAN - - - - - - - - -	567 a	.684 b
20 percent PRO-XAN - - - - - - - - -	569 a	.669 c d
20 percent PRO-XAN + lysine- - - -	557 a	.683 b c
20 percent PRO-XAN + lysine + cholesterol- - - - - - - - - - -	581 a	.676 b c d
20 percent PRO-XAN + K + Mg- - - -	578 a	.668 d

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[1] Day-old white-rock cockerels were fed a corn-soy ration for 7 days followed by the experimental rations for 19 days. There were 4 replicates of 7 chicks per replicate for each ration. Average chick starting weight, 126 grams.

[2] Means not followed by the same letter are significantly different (P = 0.05) by Duncan's multiple range test.

Analysis of the feed efficiency data showed there was no significant difference between the 5 percent PRO-XAN ration and the corn-soy ration. However, the feed efficiencies from all other rations were significantly different from these two. The feed efficiency from the 20 percent PRO-XAN diet with no additions was not significantly different from that of any of the other 20 percent PRO-XAN diets, indicating that in this PRO-XAN the lysine was essentially all available and there was no appreciable saponin present. The significant decrease in feed efficiency with increasing level of PRO-XAN very likely is due to the metabolizable energy of this PRO-XAN being less than the estimated 1180 kcal. per pound.

The moisture content of the chick excreta from this experiment is shown in table 9. During the second experimental week excreta samples were collected from each of the four replicates on each ration and the percent moisture was determined for each of these. Analysis of the data shows that the excreta moisture content from all the rations is either not significantly different from or significantly lower than that from the corn-soy control ration. The excreta moisture content appears to be related to the dietary potassium level. Consequently, from this data at least 20 percent PRO-XAN can be used in a broiler ration with no apparent diarrhea problem.

Table 9.--Percent moisture content of chick droppings, 71-154[1]

Ration	Percent moisture in droppings[2]
Corn-soy	86.2 a
5 percent PRO-XAN	84.8 a
10 percent PRO-XAN	81.5 b c
20 percent PRO-XAN	79.6 c
20 percent PRO-XAN + lysine	79.6 c
20 percent PRO-XAN + lysine + cholesterol	79.7 c
20 percent PRO-XAN + K + Mg	83.2 a b

[1] Samples were collected from each of the 4 replicates on each ration over a period of 2 hours.

[2] Means not followed by the same letter are significantly different (P = 0.05) by Duncan's multiple range test.

In summary, from both of these chick experiments we have shown that: (1) The PRO-XAN lysine appears to be essentially all available to the chick; (2) there is no detectable saponin problem in PRO-XAN; (3) dietary PRO-XAN levels of up to 20 percent cause no diarrhea; and (4) excellent chick growth can be obtained using high dietary levels of PRO-XAN. From this data it appears that PRO-XAN is a very good protein source for broiler starter rations.

A COMPARISON OF XANTHOPHYLL SOURCES

William L. Arnold, Director of Marketing,
American Dehydrators Association, Kansas City, Mo.

The broiler industry has markedly improved chick performance in the last two decades. Feed conversion has improved and days to market have decreased. For example, Dr. Leonard Dansky, Director of Research for Lipman Research Center, Augusta, Maine, reports that in 1952 they were marketing birds in 80 days averaging 3.35 pounds and requiring 3.17 pounds of feed per pound of weight. By 1965, Lipman was marketing 15 million birds. Their birds were reaching market weight in 59 days weighing 3.81 pounds and requiring only 2.03 pounds of feed per pound of weight. Since 1965, they have continued to improve overall efficiency, making them one of the leaders in the broiler industry.

Dr. Dansky feels that the biggest single factor responsible for this improvement is the development of high-nutrient density diets designed to take full advantage of the broiler chicks genetic potential. Lipman's diets contain a maximum level of high-quality animal protein and fat, and have always contained high-quality dehydrated alfalfa. Some nutritionists raise the question: Is the use of dehydrated alfalfa incompatible with the high-nutrient density diet principle? Studies were started at the Lipman Research Center to help answer the question.

The studies were conducted to evaluate: The performance of various grades of dehydrated alfalfa in high-efficiency broiler rations, and various sources of xanthophyll (high-grade dehydrated alfalfa, PRO-XAN (a concentrated alfalfa product), Ecuadorian marigold petals, and corn gluten meal) as pigmenters in high-efficiency broiler rations.

Series I

Three replicate pens of 250 straight-run broiler chicks per treatment were utilized to compare the value of test rations containing 17 percent, 22 percent, and 25 percent protein dehydrated alfalfa with a control ration void of the test material (table 1). The control diet contained 10 mg./lb. xanthophyll in the starter and finisher. Three of the treatment groups' diets contained 16 mg./lb. in the starter and 18 mg./lb. in the finisher, with dehydrated alfalfa in the starter and finisher. The other three treatment groups' diets contained 10 mg./lb. in the starter and 18 mg./lb. in the finisher with dehydrated alfalfa only in the finisher. The 17 percent, 22 percent, and 25 percent dehydrated alfalfa were added at the rate of 55, 45, and 40 pounds per ton, respectively, in the finisher diets and at the rate of 40 pounds each when added to the starter diets. The birds were marketed at 56 days of age.

When the three grades of dehydrated alfalfa were added to the finisher only, the average weights and feed conversion were equal or better than the control (table 2). When dehy was added to the starter and finisher diets, average weight and feed conversion were essentially the same as the control. No difference in performance was demonstrated among the dehydrated alfalfa products.

Visual observers concluded that all dehydrated alfalfa supplemented groups showed superior carcass pigmentation compared to the control. No difference was observed between the three alfalfa supplemented groups.

Table 3 lists some important nutrient restrictions for diets used in series I and series II experiments.

Series II

Five replicate pens of 110 straight-run broiler chicks per treatment were fed identical starter diets from 0 to 5 weeks of age (table 4). The finisher diets varied in that approximately 20 percent of the xanthophyll was supplied by 25 percent protein dehydrated alfalfa, PRO-XAN, Ecuadorian marigold petals, or corn gluten meal. The remainder of the xanthophyll requirement was supplied by corn and corn gluten meal.

Table 5 lists the performance data. These data are in conflict with those in series I in that average weight was decreased and feed required per pound of weight was increased when the xanthophyll requirement was raised from 10 mg./lb. to 16 mg./lb. in the finisher diet. However, performance was not affected as much when 3 mg. of the extra 6 mg. came from 25 percent dehydrated alfalfa, PRO-XAN, and marigold as it was when all 6 mg. came from corn gluten meal.

It was impossible to visually detect pigmentation differences in birds from the corn gluten meal, 25 percent dehydrated alfalfa, or PRO-XAN diets. All observers agreed that the birds receiving the marigold petals showed poorer pigmentation than did the birds receiving dehydrated alfalfa, corn gluten, and PRO-XAN.

Representative samples of birds from each group were compared for pigmentation using a Hunterlab D-20 color difference meter (table 6). The b-scale average readings of the breast feather tract were: (1) 23.94, control; (2) 25.84, corn gluten meal; (3) 24.56, 25 dehydrated alfalfa; (4) 24.84, PRO-XAN; and (5) 23.59, marigold petals. Treatment group 2 had yellower breasts compared to groups 1 and 5. Groups 2, 3, and 4 were not significantly different.

Dehydrated alfalfa is an important source of xanthophyll and maintains performance in high-efficiency broiler diets.

Table 1.--Experimental plan

[Market age 56 days]

Lot No.	Xanthophyll (mg./lb.)		Dehy grade (percent)		Dehy (lbs./ton)	
	Starter	Finisher	Starter	Finisher	Starter	Finisher
1	10	10	--	--	---	---
2	16	18	17	17	40(2.12 mg.)	55(2.92 mg.
3	16	18	22	22	40(3.50 mg.)	45(3.74 mg.
4	16	18	25	25	40(4.94 mg.)	40(4.94 mg.
5	10	18	--	17	---	55(2.92 mg.
6	10	18	--	22	---	45(3.74 mg.
7	10	18	--	25	---	40(4.94 mg.

Table 2.--Broiler performance as affected by level and type of dehy meal[1]

[Market age 56 days]

Lot No.	Dietary variable	Average weight, pound	Feed conversion (feed/unit wt.)	Cost per pound
1	Control - No dehy:	4.13	1.97	0.0959
	17 pct. dehy:			
2	Starter + finisher	4.14	1.99	.0969
5	Finisher only	4.21	1.94	.0945
	22 pct. dehy:			
3	Starter + finisher	4.18	2.00	.0974
6	Finisher only	4.14	1.97	.0961
	25 pct. dehy:			
4	Starter + finisher	4.13	1.99	.0971
7	Finisher only	4.23	1.94	.0945

[1] 3 replicate pens of 250 straight-run chicks per treatment.

Table 3.--Some important nutrient and ingredient restrictions for diets used in series I and II experiments

Restriction	Starter	Finisher
Metabolizable energy (cal./lb.)	1535	1560
Crude protein, percent	25.0	22.0
Calcium (minimum percent)	.86	.86
Calcium (maximum percent)	1.00	1.00
Available phosphorous (minimum percent)	.45	.41
Available phosphorous (maximum percent)	.55	.50
Animal fat (maximum percent)	7.0	7.0
Hi-protein gluten meal (maximum percent)	8.0	8.0
Poultry by-product meal (maximum percent)	2.5	2.5
Herring meal (minimum percent) Series I	4.0	3.75
Herring meal (minimum percent) Series II	4.0	3.75
Herring meal (maximum percent) Series I	7.5	6.25
Herring meal (maximum percent) Series II	7.0	5.0
Xanthophyll (mg./lb.) Series I	10.0 or 16.0	18.0
Xanthophyll (mg./lb.) Series II	10.0	16.0

Table 4.--Experimental plan

Lot No.	Xanthophyll (mg./lb.)[1] Starter	Xanthophyll (mg./lb.)[1] Finisher	Test material (Supplying 3 mg. xanthophyll in finisher)
1	10	10	- - - -
2	10	16	Corn gluten meal
3	10	16	25 percent dehydrated alfalfa
4	10	16	PRO-XAN
5	10	16	Marigold petals

[1] All xanthophyll supplied by corn and corn gluten meal except for 3 mg. of test material in finisher.

Table 5.--The effect of level and source of xanthophyll on the performance of broiler chicks[1]

[Market age 56 days]

Lot No.	Source and level of xanthophyll[2]	Average wt. (lb.)	Feed unit wt.
1 --	Control - 10 mg. xanthophyll from corn + corn gluten meal in starter and finisher	4.01	1.81
2 --	Finisher - 16 mg. xanthophyll from corn + corn gluten meal	3.89	1.84
3 --	Finisher - 16 mg. xanthophyll from corn + corn gluten meal + 25 percent dehy alfalfa meal (3 mg.)	3.95	1.84
4 --	Finisher - 16 mg. xanthophyll from corn + corn gluten meal + PRO-XAN (3 mg.)	3.96	1.84
5 --	Finisher - 16 mg. xanthophyll from corn + corn gluten meal + marigold petals (3 mg.)	3.95	1.84

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[1] 5 replicate pens of 110 straight-run broiler chicks per treatment.

[2] Birds were fed an identical starter from 0 to 5 weeks of age.

Table 6.--Instrumental measurement of skin color

	b-Scale: (Yellow color) - breast feather tract			b-Scale: Thigh		
Lot No.	Left	Right	Average	Left	Right	Average
1 ---	24.04	23.85	23.94	20.22	20.56	20.39
2 ---	25.77	25.91	25.84	23.44	22.19	22.82
3 ---	24.40	24.72	24.56	21.68	22.40	22.04
4 ---	24.92	24.75	24.84	22.98	22.90	22.94
5 ---	23.19	23.99	23.59	21.90	22.62	22.26

Significant differences (Hartley modification of range test)

Breast b-scale: 2 > 1
2 > 5
(2,4,3 not significantly different)
(4,3,1,5 not significantly different)
Thigh b-scale: 1 < 2,3,4,5
(2,3,4,5 not significantly different)

METHODOLOGY IN DETERMINATION OF XANTHOPHYLL

R. E. Knowles, A. L. Livingston, and G. O. Kohler
Western Marketing and Nutrition Research Division
Agricultural Research Service, USDA
Berkeley, Calif.

Introduction

Increasing industry interest in xanthophyll content of feed ingredients for poultry rations has spurred the development of xanthophyll analytical methods in recent years.

Before reviewing these efforts, however, let us briefly state what is desirable or essential in an analytical method for carotenoids:

1. Relative simplicity is desirable: The equipment should be simple, the time required to perform the analysis reasonable, and the work performable by technicians with reasonably brief training.

2. Accuracy: The method must measure the true content of the carotenoids which it is designed to determine. Therefore, chlorophyll contamination of eluates, improper analytical wavelength or absorptivity, and incomplete band separation on the adsorption column must be guarded against by the requirements of the method.

3. Adequate precision or reproducibility of test results must be obtainable routinely, such that real variation in the samples tested can be detected with confidence.

4. Specifically for a xanthophyll determination, the test result should give a measure of the pigmenting value of the material tested. This might be: (a) An empirical value which is consistently related to the pigmenting potency of the sample; or (b) it might be the actual content of "pigmenting xanthophylls" only.

The carotenoids present, to be analyzed, have largely determined the methodology suitable to the purpose. We may divide the carotenoids of alfalfa (fig. 1) into three general categories:

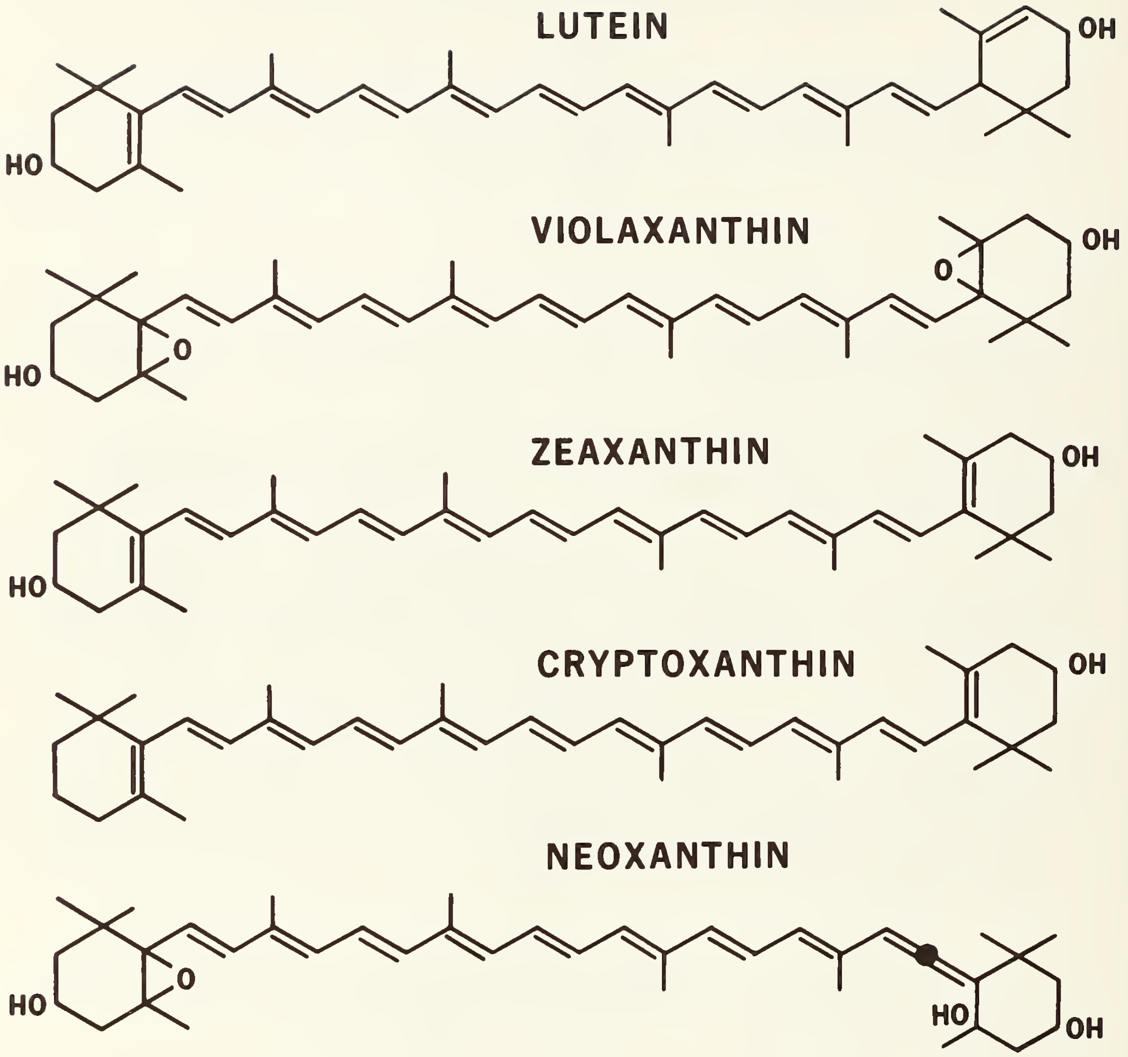

Figure 1.--Carotenoids of alfalfa.

1. Nonoxygenated, nonpigmenting carotenoids (i.e., for poultry)--the carotenes (chiefly β-carotene and its cis-trans isomers, in alfalfa).

2. Pigmenting oxycarotenoids--the hydroxy xanthophylls, both monohydroxy (β-cryptoxanthin, zeinoxanthin) and dihydroxy (lutein, zeaxanthin).

3. Nonpigmenting (or weakly pigmenting) oxygenated carotenoids--in alfalfa, chiefly violaxanthin (dihydroxy, diepoxide) and neoxanthin (trihydroxy, monoepoxide), plus possible uncharacterized "poloxy" carotenoids.

Structure of the carotenoids has definite effects upon solubility and adsorbability, and this, of course, makes possible their separation by adsorption chromatography.

We will chiefly consider column chromatography, as it is the basis for the practicable analytical methods devised for process and trading quality control. Probably the two most useful adsorbents for column chromatography of carotenoids are activated magnesium oxide and silica gel, each combined with an inert filter-aid such as Hyflo-Supercel, 1:1 by weight.

When a solution of mixed carotenoids is put onto a magnesia column, structural differences produce the following effects:

1. The nonhydroxy compounds - the carotenes - are readily eluted with weakly polar solvents such as 9:1, hexane:acetone.

2. A hydroxyl group or additional hydroxyls retard elution markedly; thus, xanthophylls as a group are easily separated from carotenes.

3. -5, 6 epoxide groups have virtually no retentive power on MgO; therefore, violaxanthin is practically not separable from lutein on a magnesia column.

4. Ester groups in carotenoids are considerably less strongly adsorbed than hydroxyls, so that di-esters will elute before dihydroxys and may even be mixed with monohydroxy carotenoids on the column; hydrolysis is therefore necessary before chromatography to prevent such mixing.

5. The β-ionone ring of carotenoids is somewhat more strongly adsorbed on magnesia than the α-type ring; thus, zeaxanthin can, with care, be separated from the faster moving lutein.

On a Silica Gel-G column, these structural effects are observed:

1. The presence of hydroxyls in carotenoids does not increase retention relatively as much as in the case of MgO. Some interesting consequences are:

   a. A less polar solvent, as 96:4, hexane:acetone, must be used for carotene elution, to avoid eluting monohydroxy compounds also.

b. Monohydroxys, however, are more readily separated on silica gel from dihydroxy xanthophylls.

c. Epoxy -5, 6 groups confer some additional retentive power to hydroxy carotenoids, which is not discernible on MgO. Therefore, on a silica column, violaxanthin is separable from lutein, more or less.

2. α- and β-ring carotenoids, as lutein and zeaxanthin, are not well-resolved on silica gel.

These, then, are some of the limitations, as well as capabilities, of adsorption chromatography.

Review of Recent Methods Development

In 1964, the new ADA Technical Advisory Committee selected four proposed xanthophyll analytical methods and conducted two preliminary collaborative trials. However, these first results were too poor to merit statistical analysis; none of the methods demonstrated good enough precision to recommend it as an official AOAC method.

The following year one of these methods ("Referee"), revised by Dr. Quackenbush of Purdue University so as to specify elution of xanthophylls with 1:1, hexane:acetone, was added to the official method for carotene in the check sample analysis[1], and was subsequently tested by the collaborators for several years. The standard deviations and coefficients of variation of the xanthophyll results reported were continually high in comparison with carotene results.

In 1966 and early 1967, a method for carotene and xanthophyll in alfalfa submitted by Dr. Kohler of this laboratory (3) was tested by seven collaborators under ADA auspices. The method required no extract-washing step, and it controlled chlorophyll by a simple potassium hydroxide treatment of the extract. Total xanthophylls, including epoxide xanthophylls, were eluted by this procedure, although the latter were discounted to a certain degree by the analytical wavelength used. This method was distributed to the ADA check sample collaborators in 1968 and varying numbers of them submitted carotene and xanthophyll results obtained by this analysis, along with their usual reports, during that year and the next. Somewhat better precision, generally, was obtained in the xanthophyll analyses by this "WRRL-1967" method.

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[1] Collaborative Check Sample Committee of the Association of American Feed Control Officials: James P. Minyard (Chairman) State Chemist of Mississippi, Drawer CR, State College 39762.

At the last Technical Advisory Council meeting, Lyle Livingston pointed out that a special analytical procedure which excludes measurement of epoxide xanthophylls would be desirable for prediction of pigmentation potency of materials high in epoxide xanthophylls, such as freeze-dried or certain sun-cured alfalfas. Such a method has since been devised in this laboratory and published in 1969 (6). It will be described later.

The ADA check sample program had continued, meanwhile, with little improvement in precision. However, with the last sample distributed in 1968 a new method, developed by Dr. Quackenbush and coworkers, was substituted. This method had been evaluated earlier in the year by 11 collaborators. Their results and the new method, which I shall designate as the "tentative AOAC method," were published in 1970(8,9). This method employs a special extracting solvent - hexane:acetone:ethanol:toluene - and a Silica Gel-G column which separates, besides carotene, monohydroxy from dihydroxy xanthophylls, and is intended to retain epoxide xanthophylls and "polyoxy" compounds on the column. A value termed "dihydroxy pigment equivalent" is obtained and proposed as an indicator of pigmentation potency of the sample. (This method also provided for the optional determination of total xanthophyll, by chromatography of the same extract on a magnesia column, as per the WRRL method, using the same eluants.)

It was indicated that this method was suitable also for carotenoid analysis of corn grain and mixed feeds containing cornmeal or corn gluten meal. The analytical method often used for corn, e.g. in pigmentation comparisons with dehydrated forages, has been that of the Corn Industries Research Foundation (10). However, due to the absorptivity for the xanthophyll fraction specified in that method, it has yielded xanthophyll values which are more than 30 percent high. The tentative AOAC method, applied to corn analysis, offered a welcome alternative to that confusion in the data.

When it became apparent that the WRRL method often yielded lower total xanthophyll values than the new tentative AOAC total xanthophyll method, the matter was investigated here. It was found that the addition of 0.5 ml. of water to the WRRL extracting solvent before overnight soaking significantly increased xanthophyll extraction in dehydrated alfalfa. This modification has therefore been adopted for analysis of heat-dehydrated alfalfa, and is described in a publication this year (5). This method will be designated "WRRL-1971" during this presentation.

Having surveyed chronologically the recent development of xanthophyll methodology, let us consider the two most promising analytical procedures in somewhat greater detail.

1. The WRRL approach to devising a method for a valid estimation of the pigmenting value of a feed sample has been as follows:

   a. Perform as complete extraction of the plant pigments as possible. I have mentioned the improvement in extraction when 0.5 ml. H_2O was added to the 7:3, hexane:acetone extractant. Table 1 demonstrates the increased extraction thus achieved, especially in whole dehydrated meal (freeze-dried or sun-dried alfalfa does not require this treatment).

Table 1.--Comparison of methods of analysis for xanthophyll in alfalfa products

Product	WRRL-1967	WRRL-1971[1]		Tentative AOAC
	Mg./lb.	Mg./lb.[2]	Mg./lb.[3]	Mg./lb.
Dehydrated whole meal - - - - - - -	155.5	180.1	182.9	181.2
Ditto- - - - - -	158.2	164.0	167.1	170.0
Ditto- - - - - -	139.8	160.3	163.5	159.7
Ditto- - - - - -	151.7	153.3	157.5	157.8
Ditto- - - - - -	233.5	253.0	260.6	252.0
Ditto- - - - - -	269.3	264.0	267.2	269.5
Dehydrated leaf meal	142.5	153.7	158.6	166.4
Ditto- - - - - - -	173.9	175.9	179.8	183.5
Dehydrated stem meal	71.1	71.7	73.3	74.6
Ditto- - - - - - -	104.2	120.9	122.6	125.5
Sun-dried alfalfa hay - - - - - - -	90.1	84.9	86.4	87.5
Ditto - - - - - -	112.8	109.5	111.5	110.9

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[1] Water added to extraction solvent.

[2] Measured at 475 mμ.

[3] Measured at 474 mμ.

b. Isolate the total xanthophyll content of the sample. In order to accomplish this, chlorophyll is excluded by the methanolic KOH treatment followed by addition of a small amount of water. Table 2 indicates the low chlorophyll content in the WRRL-1967 analyses in which the KOH treatment was used. (No KOH was used in the other methods; the referee method required a water-wash step.) When the WRRL-1971 modified method of extraction is used with 0.5 ml. H_2O in the extractant, a holding period of 30 minutes following addition of KOH and 60 minutes following a further addition of water is specified; chlorophyll control is then assured. Separation of total xanthophylls on a magnesia column by elution with 80:10:10, hexane:acetone:methanol is then simple and relatively foolproof.

Table 2.--Chlorophyll control in analysis for carotene and xanthophyll in dehydrated alfalfa[1]

Method	Carotenes (A at 435 mμ)	Xanthophylls (A at 475 mμ)	Chlorophyll (A at 660 mμ)
	Mg./lb.	Mg./lb.	
Referee - - - - -	96.8	102.4	0.006
WRRL-I - - - - -	103.9	145.9	.294
WRRL-II - - - - -	102.2	140.9	.034
WRRL-1967 - - - -	108.5	137.5	.016

[1] Values are average of 4 replicates.

c. Measure the total xanthophyll fraction spectrophotometrically and calculate as lutein-equivalent xanthophyll; this yields a value which has been useful with regard to poultry pigmentation. This will be discussed in the next presentation.

d. However, it is a simple procedure then to treat this xanthophyll eluate with ethanolic hydrochloric acid according to the WRRL-1969 procedure of Livingston, Nelson, and Kohler. This converts the -5,6 epoxide xanthophylls to their -5,8 epoxide isomers. A shift of 35 mμ toward the violet in the long-wavelength peak position of violaxanthin occurs, while a similar isomerization of the single epoxide group of neoxanthin causes a downward shift of approximately 18 mμ in its long-wavelength peak position. Of significance to this procedure is the effect upon absorbance at 475 mμ. This is illustrated in figure 2. Following isomerization, violaxanthin (now auroxanthin) has insignificant absorbance at 475 mμ, neoxanthin (-5,8) has 2 to 4 percent of its previous A_{475}, and lutein (due to cisisomerization) has 89 percent of its all-trans A_{475}. By using an absorptivity (210) which corrects for acidified lutein in the calculation, the content of chiefly nonepoxide (NEX) pigmenting xanthophyll can be calculated. (Previously published data (6) indicating no significant difference between total xanthophyll and NEX analyses for dehydrated alfalfa were based upon somewhat lower total xanthophyll extraction by the WRRL-1967 method and an arbitrary use of 196 as the absorption coefficient for NEX calculation, in order to correlate these results. However, with the improved extraction by the WRRL-1971 total xanthophyll method, the 210 absorption coefficient should be used for calculation of nonepoxide xanthophylls.) As illustrated in table 3, this pigmenting xanthophyll value is lower, generally significantly so, than the total xanthophyll value for dehydrated alfalfa meals. The difference reflects chiefly the amount of violaxanthin or neoxanthin surviving the dehydration process.

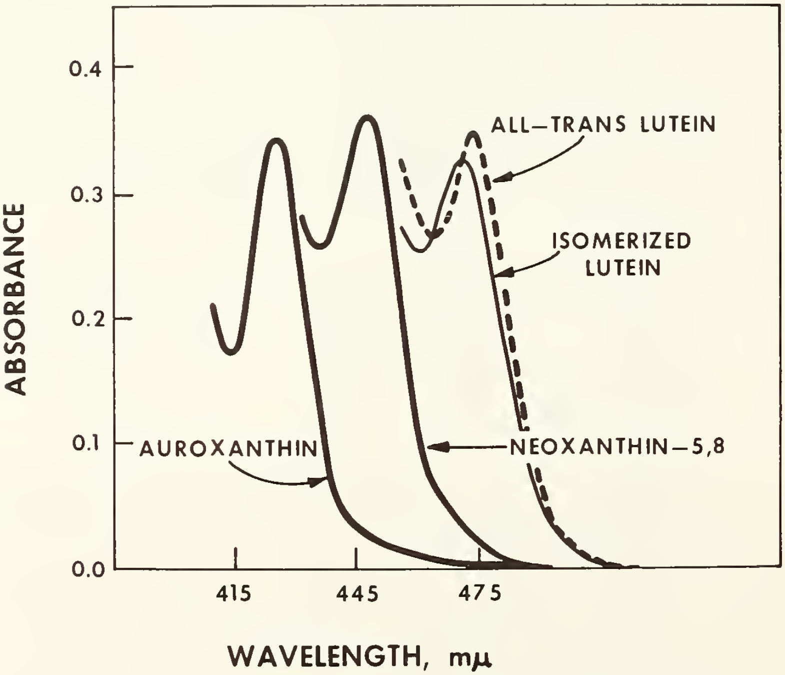

Figure 2.--Absorbance at 475 mμ following HCl-isomerization of violaxanthin, neoxanthin, and all-trans lutein.

Table 3.--Analysis of whole dehy alfalfa by the WRRL-1971 method[1]

Sample No.	Carotene	Total xanthophyll	Pigmenting xanthophyll
	Mg./lb.	Mg./lb.	Mg./lb.
908	132.5	190.4	172.5
909	61.1	110.9	100.0
960	115.6	147.1	142.9
961	80.8	120.5	113.1
997	101.8	147.1	136.4
998	118.1	151.2	141.8
49	121.5	165.6	147.8
51	106.3	153.3	142.5
111	115.7	162.6	158.6
112	114.6	156.6	150.7
183-1	53.2	117.0	109.8
183-2	106.1	125.6	123.6
183-3	95.6	126.3	125.1
148-2	52.3	72.2	68.9
150-1	98.8	99.9	97.3
151-1	59.8	101.5	83.5
121	133.4	168.1	163.5
37	151.6	181.4	166.2

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[1] Duplicate analyses.

2. A different approach to estimation of only the pigmenting xanthophylls of plant materials is to separate them on the chromatographic column from epoxide and "polyoxy" compounds. This is the technique of the 1970 tentative AOAC procedure of Quackenbush et al. (9), which employs a Silica Gel-G column. It also separates monohydroxy pigments, assigns them a pigmentation value equal to 50 percent that of lutein, and calculates a dihydroxy pigment equivalent (DHPE). This figure is considered to be well-correlated with the poultry shank pigmenting value of the plant material analyzed.

It is of interest to compare this DHPE value with WRRL pigmenting xanthophyll values determined on the same samples. Table 4 presents several such comparisons. At the 95-percent confidence level, eight of these pairs of values are not significantly different; the rest are evenly divided in higher and lower results by the two methods.

The tentative AOAC method specifies an ingenious one-step aqueous wash of the extract in the extraction flask, and defines the extract volume as 50 ml. Attempts to verify this volume in our laboratory have indicated that it is approximately 47 ml. instead. Since calculation of the aliquot taken for chromatography

Table 4.--Analysis of whole dehy alfalfa[1]

Sample No.	WRRL-1971 (Pigmenting xanthophyll)	Tentative AOAC (Dihydroxy pigment equivalent)
	Mg./lb.	Mg./lb.
908	172.5	177.9
909	100.0	106.9
960	142.9	147.8
961	113.1	123.3
997	136.4	139.4
998	141.8	152.2
49	147.8	159.4
51	142.5	142.7
111	158.6	147.3
112	150.7	143.3
183-1	109.8	110.6
183-2	123.6	121.2
183-3	125.1	119.8
148-2	68.9	67.8
150-1	97.3	90.1
151-1	83.5	86.6
121	163.5	151.3
37	166.2	169.0

[1] Duplicate analyses.

is based upon this figure, it seems that an error of about 6 percent on the high side is introduced here. The method omits all the neoxanthin from the category of pigmenting xanthophylls although Kuzmicky et al. (4) found that neoxanthin had about 8 percent broiler skin pigmenting potency, compared with lutein. The tentative AOAC method is also somewhat more lengthy and complicated than the WRRL-1971 (pigmenting xanthophyll) method. A very probable source of error in the method is the indefinite separation between the eluting dihydroxy pigment band and the violaxanthin above it, which must not be eluted. Considerable individual judgment is required here, and the matter is complicated by lutein and zeaxanthin cis-isomers present in this part of the silica column. It is quite possible to elute additional pigment with the "dihydroxy pigment," giving erroneously high results.

Special Purpose Methods for Xanthophyll Determination

1. Thin layer chromatography has proven useful for more detailed separation of carotenoids.

   a. One-dimensional TLC on a 1:6, Silica-G:lime plate, the method of Nelson and Livingston (7), applying the sample of concentrated whole plant extract as a streak along one edge of the plate, and developing in 2 percent butanol:benzene, permits separation of at least six xanthophyll and carotene bands. This technique has been invaluable in studying, for example, the effect of storage on xanthophylls in dehydrated forages.

   b. Two-dimensional, two-adsorbent TLC, a procedure published by us recently (1), carried out on an MgO:Kieselguhr plate having a Silica-G:lime strip along one edge, for the first dimension development, permits a more detailed separation of carotenoids in forage extracts. Carotene, individual monohydroxys, all the major xanthophylls and their cis-isomers, and epoxy -5,6, and -5,8 isomers can be isolated on the plate. They can also be eluted and their visible spectra determined. This is a time-consuming method, but valuable for certain investigations.

2. A reflux method of extraction of carotenes and xanthophylls from dried forages is being developed. Such a method would undoubtedly fill a specific need in the industry for process control purposes.

A 60-minute reflux extraction of dehydrated alfalfa meal with 7:3 hexane:acetone plus 0.5 ml. H_2O, at 60° C., is followed by addition of a small amount of hexane. Methanolic KOH is then thoroughly mixed with the sample and further treatment and chromatography are approximately the same as in the WRRL 1971 method. One analysis can be completed in approximately 2 hours and a battery of six samples and equipment can be conveniently managed by one analyst. The results appear to be quite acceptable, particularly for a method requiring limited time for completion. Details will be presented in a forthcoming publication (2).

Conclusion

We believe that the analytical tools for dehydrated forage production control and research have been much improved since the last meeting of this group and should aid the industry to produce alfalfa products of assured high quality, particularly with regard to xanthophyll content.

Acknowledgment

We wish to express grateful acknowledgment to Mrs. Katie Summers for much technical assistance rendered.

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CORRELATION OF CHEMICAL METHODS AND BIOLOGICAL ASSAYS FOR XANTHOPHYLL

A. L. Livingston, D. D. Kuzmicky, G. O. Kohler, and R. E. Knowles
Western Marketing and Nutrition Research Division, Agricultural Research Service, USDA, Berkeley, Calif.

The quality and the acceptance of poultry products is usually associated by the consumer with the color of the product. The nutritionist and feed formulator therefore seeks feed ingredients which will supply sufficient pigment using the computer to make least-cost feed formulations. I shall share with you some of the studies we have conducted in providing the feed formulator information that will enable him to correlate chemical analysis of xanthophyll sources, such as dehy alfalfa, with expected poultry pigmentation or biological results.

Xanthophyll pigmenters may be obtained from either natural sources such as dehy alfalfa, PRO-XAN, grass meal, corn gluten meal, marigold petals or from synthetic carotenoid preparations. There are three naturally occurring xanthophylls which make up about 90 percent of the pigmenting xanthophyll in the alfalfa, corn, and marigold sources. These are lutein, zeaxanthin, and cryptoxanthin (figure 1). Three synthetic xanthophylls are also

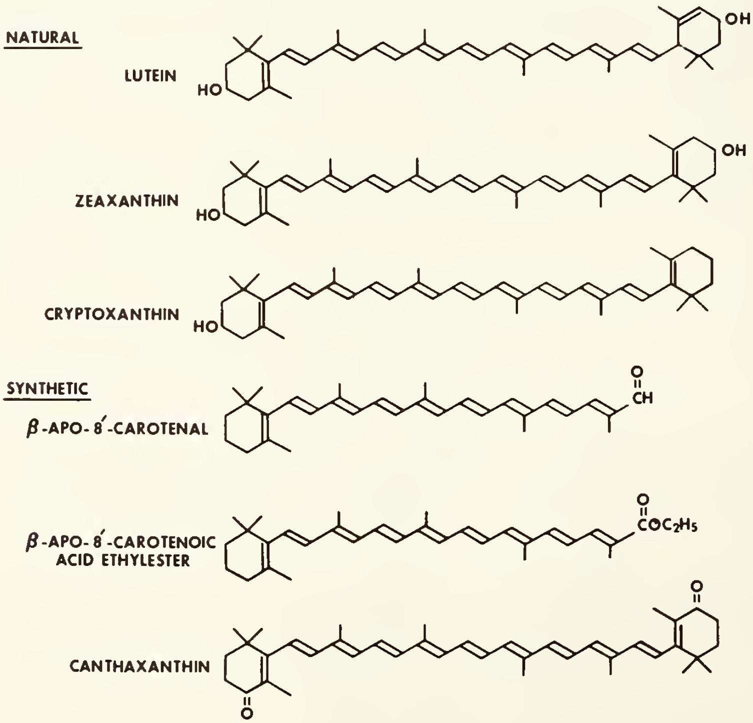

Figure 1. Structural formulas of poultry pigmenters.

fed in poultry rations in Europe and South America to give the desired dark orange egg yolks and broiler skin which the consumer seems to prefer in these countries. These are β-apo-8'-carotenal, β-apo-8'-carotenoic acid ethyl ester, and canthaxanthin. None of these synthetics is presently being used in poultry feeds in the U.S., although β-Apo-8'-carotenal and canthaxanthin are added as color agents to salad dressings and other foods (1, 2). Both of these synthetics have also been isolated from natural sources.

The color imparted to broiler skin, egg yolk or to a solution by a xanthophyll is closely related to its structure, and may be associated with the number of conjugated double bonds in the chain of the molecule. Thus lutein has 10 conjugated double bonds, zeaxanthin 11, and canthaxanthin 13. Therefore, chemically lutein is able to produce a yellow color in products, zeaxanthin will give orange-colored products, and canthaxanthin due to 13 conjugated double bonds gives products of red color.

Now that we have considered the chemical nature of the principal pigmenters and their pigment potential we might consider the sequence of evaluating the effectiveness of a particular xanthophyll as a poultry pigment source.

There are four main aspects to this. They are: analysis of the pigment source, availability to poultry, utilization by the poultry, and analysis of the product.

Mr. Knowles has just described the first aspect, the chemical analysis of the xanthophyll feed source. Since most natural sources contain more than one xanthophyll and not all are pigmenters, this can be complex. Our studies have previously found neoxanthin and violaxanthins noneffective pigmenters (5) and therefore developed a procedure which excluded them from analysis. This has been known as the nonepoxide xanthophyll or NEX procedure (7).

The stability of xanthophyll in the feed mixture is also very important. This is usually accomplished by the addition of an antioxidant such as ethoxyquin to the dried forage source prior to incorporation into the mixed feed.

Another important aspect in correlating analysis and biological activity is that of availability or adsorbability. We have previously found that the availability of xanthophyll in dehy alfalfa leaf or whole meal was increased by physically modifying the meal, in this case pelleting and regrinding (3).

As shown in table 1 we have also found that xanthophyll in extracts of both dehy alfalfa and corn gluten meal were more available for pigmenting broiler skin than was xanthophyll in the whole meal. In this instance the xanthophyll extracts were very carefully prepared so as to include the same proportion of xanthophylls as the whole meal.

Table 1.--Relative broiler pigmentation of corn gluten and dehy alfalfa

Supplement	Relative skin pigmentation potency[1]
Pure lutein - - - - - - - - - - - - -	1.00
Dehy alfalfa- - - - - - - - - - - - -	.58
Dehy alfalfa extract- - - - - - - -	.72
Corn gluten - - - - - - - - - - - - -	.52
Corn gluten extract - - - - - - - -	.72

[1] Calculated by Slope Ratio Assay.

We might next consider utilization of the carotenoid by the bird. An example is β-carotene which is very similar in structure to zeaxanthin (except for two oxygens) but is a non-pigmenter. Similarly the xanthophyll cryptoxanthin contains one hydroxyl and has been reported by Quackenbush et al.(9) to be half as effective as zeaxanthin as a pigmenter, apparently due to half of the molecule being converted to vitamin A during digestion.

Another example of utilization by the bird is β-apo-8'-carotenal compared to β-apo-8'-carotenoic ethyl ester. The free aldehyde carotenal is a fairly effective egg pigmenter but a very poor broiler skin pigmenter, while the ester has proved to be an effective broiler skin pigmenter.

The fourth important factor in considering effectiveness of a pigment source is measurement of the particular xanthophyll in the poultry product. This has been accomplished by a variety of means. One is by chemical extractions of either egg yolks or a part of the broiler skin and measurement of the extract at a suitable wavelength. A second means is that of visually comparing the color of the egg yolk or broiler skin with a color rotor or color fan. This has several problems, one of which is that the color fan does not always contain the same proportion of yellows and reds as does the tissue or yolk being measured. Thus, if you compare the effectiveness of pure lutein and canthaxanthin (one imparts a yellow and the other a red pigment to broiler products), what should be the composition of the fan employed in measuring their pigment potency? Another problem is that although the fan may be standardized, the lighting conditions under which the product is scored are not standardized and great variations in scoring are found between laboratories.

A more recent procedure in the scoring of poultry products is the use of a color-measuring instrument such as a color difference meter. This has advantages over chemical and visual scoring in that it is less time consuming than the chemical procedure and more objective than the visual method.

To demonstrate the problem natural feed supplements may have in scoring high with a color fan, we might compare the Roche color fan with the color difference meter to ascertain what changes occur in the makeup of the fan with increasing fan numbers. Employing the color meter we compared the changes in lightness, redness, and yellowness with fan numbers (table 2).

Table 2.--Color meter reflectance measurements of Roche color fan (1965)[1]

Fan No.	L (Luminosity)	a (Redness)	b (Yellowness)
1	55.5	-4.1	30.9
2	55.0	-3.6	32.2
3	54.4	-2.8	33.5
4	54.0	-2.0	34.8
5	53.2	- .8	35.3
6	52.5	.7	35.7
7	51.8	2.2	36.0
8	51.6	3.5	36.2
9	51.0	5.3	36.2
10	50.7	7.4	36.5
11	49.1	8.9	34.7
12	47.6	11.4	33.5
13	46.3	14.0	31.9
14	44.6	16.2	30.3
15	42.9	17.9	28.5

[1] Standardized with tile No. 2005 - L = 56.3, a = -3.6, b = 24.2.

In addition the color fan actually was designed to measure changes in egg yolk color which contains more gloss than broiler skin. It is apparent that the amount of yellowness levels off and actually decreases at the higher fan numbers as described by Vuilleumier (10).

As I mentioned in my introduction, the chemical makeup or number of conjugated double bonds in the molecule determines the color imparted by a xanthophyll. We would therefore not expect the same amount of redness to be imparted to a poultry product by a given amount of lutein as by zeaxanthin. To demonstrate this theory and apply it to reflectance measurements, solutions of lutein, zeaxanthin, and β-apo-8'-carotenal were prepared and strips of filter paper were dipped into solutions of the respective xanthophyll. After drying under an inert gas the color reflectance of each strip was measured on the color difference meter. As shown in figure 2, it is apparent that lutein contains more yellow at a given concentration than does either zeaxanthin or β-apo-8'-carotenal.

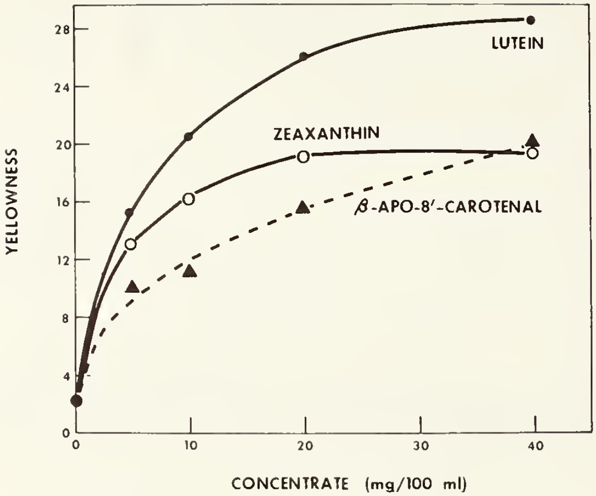

Figure 2. Color reflectance of carotenoids on paper.

Conversely, we see at varying levels that zeaxanthin and β-apo-8' impart more red than lutein to the strips of filter paper (figure 3).

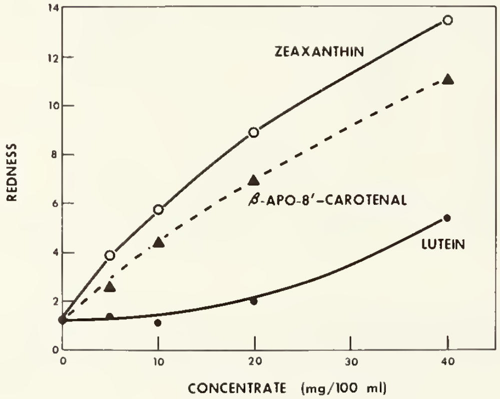

Figure 3. Color reflectance of carotenoids on paper.

From this physical-chemical demonstration we might expect that poultry rations containing mostly lutein would give more yellow to the broiler skin or egg yolk while zeaxanthin would give more red. Mixtures of the two would be expected to give yellow-orange colors.

We have also utilized the color meter in the analysis of broiler shank skin following feeding trials in which dehy alfalfa, corn gluten, and the synthetic xanthophylls were included in the diet. Increasing levels of the supplements, dehy alfalfa and corn gluten meal, were found to contribute similar amounts of yellowness to broiler skin. However, in terms of redness we found that dehy alfalfa was the lesser source of red pigment. Both the carotenoic ester and corn gluten meal contributed considerable redness, while the addition of canthaxanthin to the dehy alfalfa greatly increased the amount of red in the broiler skin.

Last year the ANRC conducted a collaborative broiler pigmentation study regarding chemical and biological correlations in the pigmentation of broiler skin. One of the principal objectives was to compare chemical methods for predicting pigmentation of poultry. The results were reported in the Feb.-Mar. issue of Feedstuffs, 1971. The experiment was supervised and reported by Dr. Marusich. Our lab participated in the study as proposed and, in addition, actually increased the number of chemical and biological parameters in an effort to gain more information on this difficult subject.

In this study the pigment supplements were analyzed by the tentative AOAC procedure of Quackenbush (8) as well as the procedures of this lab (6, 7). The corn gluten was also analyzed by the Corn Industries method. The results as shown in table 3 indicate that the tentative AOAC procedure for total xanthophyll and the WU-total xanthophyll (4) yield comparable analysis for dehy alfalfa. Also the DHPE and NEX-xanthophyll procedures yield comparable results. However, the Corn Industries method gave about 30 percent higher xanthophyll analysis for corn gluten meal than did the DHPE procedure, resulting in a lower apparent availability of the xanthophylls in the corn gluten meal.

Table 3.--Analysis of pigment supplements (milligrams xanthophyll per pound)

Xanthophyll method	Dehy alfalfa	Corn gluten meal
WU-total xanthophyll - - - - -	156.3	- - -
WU-NEX[1] - - - - - - - - - - - -	140.4	- - -
Tentative AOAC-total xanthophyll	154.2	- - -
Tentative AOAC-DHPE - - - - -	137.4	134.6
Corn Industries - - - - - - - -	- - -	197.5

[1] Employed Extinction Coefficient of 210.

In this study the footpads of the broilers were analyzed visually by employing the Roche color fan and chemically by extraction of the toe web punches. The results are presented in tables 4 and 5.

Table 4.--Visual pigmentation potency relative to lutein[1]

Xanthophyll method	Dehy alfalfa	Corn gluten meal
	Percent	Percent
WU-total xanthophyll	53	--
WU-NEX	59	--
Tentative AOAC-total xanthophyll	54	--
Tentative AOAC-DHPE	61	73
Corn Industries	--	50

[1] $\frac{\text{Visual score from supplement/mg. pigment per lb. of ration}}{\text{Visual score from lutein/mg. lutein per lb. of ration.}}$

Table 5.--Chemical pigmentation potency relative to lutein from toe web analysis[1]

Xanthophyll method	Dehy alfalfa	Corn gluten meal
	Percent	Percent
WU-total xanthophyll	57	--
WU-NEX	63	--
Tentative AOAC-total xanthophyll	57	--
Tentative AOAC-DHPE	64	61
Corn Industries	--	42

[1] $\frac{\text{Toe web score from supplement-control/mg. pigment/lb. of ration}}{\text{Toe web score from lutein-control/mg. lutein/lb. of ration.}}$

Both the WU method for total xanthophyll and the tentative AOAC method for total xanthophyll gave equivalent pigmentation potencies for dehy alfalfa as measured visually with the Roche color fan or by chemical extraction and measurement of the toe web xanthophylls. Likewise the WU-NEX procedure and the tentative AOAC-DHPE method gave equivalent pigmentation potencies for dehy alfalfa. The corn gluten meal and dehy alfalfa when analyzed by the DHPE procedure were equal in potency when the toe webs were extracted and the xanthophylls measured chemically. However, when the toe webs were visually scored the tentative AOAC-DHPE procedure gave poor correlation for the relative pigmentation potencies of dehy alfalfa and corn gluten meal (61 compared to 73 percent). The Corn Industries procedure gave a potency of 50 percent compared to pure lutein, when the results were scored visually, agreeing well with the WU-total xanthophyll procedure of 53 percent. However, when the broiler skin was chemically scored, the Corn Industries method appears to be a poor estimator of pigmentation potency.

It is probably very significant in this study that a medium level of pigment was included in the diet since the viaual color fan scores indicate a range where lutein would be most effective. At higher levels, redness would have been more significant and zeaxanthin would contribute more than lutein to the visual scores. Therefore the corn gluten meal which contains a greater proportion of zeaxanthin than dehy alfalfa would have scored higher when evaluated by the color fan.

In summary then the correlation of chemical analysis and biological activity is a very complex subject. It involves not only a chemical analysis of the pigment source in the ration and measurement of the color of the poultry product but the chemical constitution of the xanthophylls, levels fed in the diet, and the relative availability and utilization by poultry of the particular pigment supplement.

Finally, a most important factor is what the consumer is seeking when the product is purchased. The feed formulator must seek to answer all of the questions and at the same time incorporate the least-cost pigment supplement in the ration. Although we don't have all the answers, progress has been made in obtaining correlations between chemical analysis and biological pigmentation.

Acknolwledgment

The authors gratefully acknowledge the analytical assistance of Mrs. K. Summers.

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NUTRITIONAL, PIGMENTING, AND STABILITY EVALUATIONS OF X-PRO

Hobart R. Halloran

Halloran Research Farm, Inc., Modesto, Calif. 95351

X-PRO is a high xanthophyll, high protein dehydrated alfalfa meal produced from alfalfa juice. It was developed jointly by Batley-Janss Enterprises, Inc., of Brawley, Calif., and the Western Regional Research Laboratory, Agricultural Research Service, USDA. Batley-Janss is producing X-PRO commercially.

We conducted ten chick and hen tests since 1968 (2). Three types of samples were evaluated: (1) A pilot-plant sample produced at WRRL. (2) A pilot-plant sample produced at the Batley-Janss plant using the WRRL portable dryer. (3) Production samples from the Batley-Janss dehydrator at Brawley, Calif.

The xanthophylls of the samples analyzed 600 to 900 mg./kg. and the protein 40 to 50 percent. The amino acid profile of the X-PRO protein is similar to that of soybean protein.

Three growth tests were conducted with commercial broiler cockerels. X-PRO levels from 1.2 to 6.4 percent were fed. There were no detrimental effects on weight gains or feed conversions.

In a protein-quality test at a suboptimal level, X-PRO protein successfully replaced half of the soybean protein in a corn-soy ration. X-PRO was fed at 8.8 percent of the formula.

Broiler pigmentation studies showed equivalent availability of the xanthophylls from X-PRO and from 19 percent and 20 percent protein dehydrated alfalfa meals.

A laying hen test with graded xanthophyll levels showed equal yolk color from xanthophylls of X-PRO and 20 percent protein dehydrated alfalfa meal.

Canthaxanthin supplementation of alfalfa xanthophylls for broilers resulted in improved Roche color scores. The gizzard fat was evaluated at the same time as the carcass color. Equal Roche color fan scores resulted. This technique is especially useful when broilers are processed at scalding temperatures.

The xanthophylls of a marigold meal sample were compared with the X-PRO xanthophylls in producing yolk color. Marigold xanthophylls had 54 percent the availability of the X-PRO xanthophylls based on a Roche color score of the yolks. The percent availability of the marigold xanthophylls was 58 percent based on the analyses of the xanthophylls in the yolks.

All analyses of the X-PRO and dehydrated alfalfa meal samples were conducted using the method of Kohler et al. (3). The marigold xanthophylls were determined using the method developed by the Corn Industries Research Foundation (4). The use of different analytical methods will result in different percent availability for the marigold xanthophylls.

X-PRO xanthophyll stability studies were conducted at seven temperature and humidity conditions for 12 months. An example is shown in the results from feed mill storage (35°-111°F.) of X-PRO samples in double kraft bags. The xanthophyll retention after 1, 3½, 6, and 12 months was 96, 90, 85, and 77 percent, respectively. Six months mill storage in a laminated paper sack resulted in 88 percent xanthophyll retention.

Computer studies on X-PRO were conducted in conjunction with Harry J. Konen & Company, Houston, Tex. Parametric linear programming techniques as developed by Halloran (1) and Taylor et al. (5) were used to determine the dollar value of X-PRO.

Acknowledgments

Batley-Janss Enterprises, Inc., Brawley, Calif., for sponsorship of all studies reported; Harry J. Konen & Company for parametric linear programming; New Jersey Feed Laboratory for xanthophyll analyses; Western Regional Research Laboratory, Agricultural Research Service, USDA--E. M. Bickoff, George O. Kohler, and A. L. Livingston for xanthophyll analyses, samples, and invaluable assistance.

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STABILITY OF PIGMENTS IN ALFALFA PROTEIN CONCENTRATES

S. C. Witt, R. R. Spencer, E. M. Bickoff, and G. O. Kohler
Western Marketing and Nutrition Research Division, Agricultural
Research Service, USDA, Berkeley, Calif.

By now, all of you know what PRO-XAN is, and that we consider it a much more suitable xanthophyll supplement for poultry rations than conventional pigmentation sources. Xanthophylls are the carotenoids which pigment egg yolks and chicken skin. The problem is that they are readily oxidized into compounds that are non-pigmenters. Therefore, means of preventing or retarding oxidative losses is really what xanthophyll stability is all about. This is extremely important for a product that is being sold for its xanthophyll content. Means of retarding these losses have been worked out on dehy. Largely due to the efforts of people at WRRL, the Food and Drug Administration approves the use of the antioxidant ethoxyquin on alfalfa and in poultry rations. Much of the dehy produced commercially in this country today is treated with ethoxyquin, either alone or in combination with a small amount of oil. This and inert gas storage are the two means used by the dehydration industry to improve xanthophyll stability during storage.

What we were interested in, in our studies, was how the factors that affect xanthophyll stability in dehy affect xanthophyll stability in PRO-XAN. The six factors we investigated were natural antioxidants, ethoxyquin, added oil, product moisture level, storage temperature, and air-free storage. Most of this work was done with drum-dried PRO-XAN. This is because it was not until this study was nearly completed that we found that hot air rotary driers like those used commercially to dehydrate alfalfa were the best way to dry PRO-XAN on a commercial scale. Storage studies are presently underway on the commercial product. At the end of this presentation, I will discuss the differences in the stability between rotary air-dried PRO-XAN and drum-dried PRO-XAN that show up after 4 weeks of storage.

Unless stated otherwise, the various tests were carried out at 100° F., samples were stored in open containers, and ethoxyquin concentration was 0.125 percent.

Alfalfa contains its own natural antioxidants which protect the xanthophylls. In fact, there are two types of natural antioxidants in alfalfa, those which are fat soluble and those which are water soluble. The main ones are the tocopherols, which are fat soluble. And more important, they are heat labile. Livingston and coworkers (4) have shown that tocopherol is partially destroyed when alfalfa is dehydrated. This apparently also happens during drum-drying. As shown in figure 1,

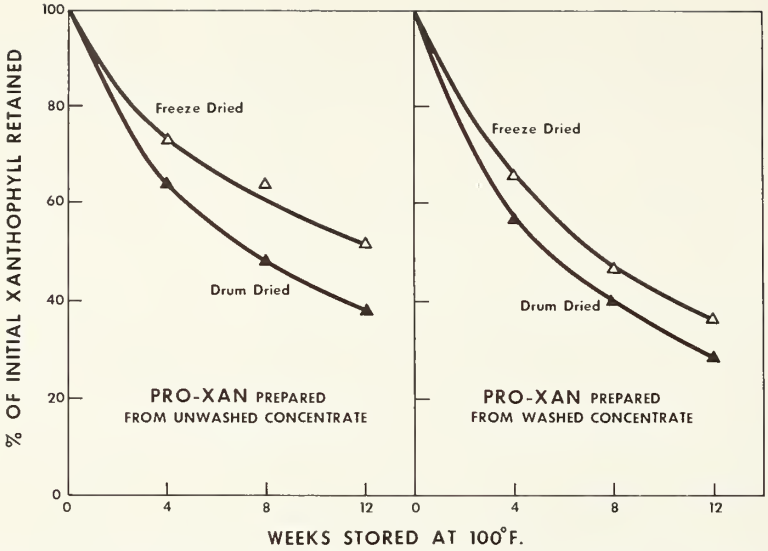

Figure 1.--Effect of freeze drying and drum drying on xanthophyll stability during open storage

freeze-dried PRO-XAN is more stable than drum-dried PRO-XAN. Freeze-drying is mild enough not to destroy the natural antioxidants of whole alfalfa, so it shouldn't affect them in PRO-XAN. This greater stability of freeze-dried material was true both for normally prepared PRO-XAN as well as PRO-XAN that had been washed before drying to remove the water soluble constituents.

Bondi and coworkers (1) in Israel have shown that the water-soluble antioxidants in alfalfa are not heat labile. However, the amount of them present in PRO-XAN depends on how well the concentrate is drained prior to drying. This is because the brown juice that is drained off contains the water-soluble constituents from the alfalfa, which would include the water-soluble antioxidants. The concentrate we prepare in our pilot plant contains about 80 percent moisture, which is equivalent to about 25 percent water solubles on a dry-weight basis. We wanted to know what effect removing more or less brown juice would have on xanthophyll stability. Therefore, we water washed some wet concentrate and added 12-1/2, 25, or 37-1/2 percent water solubles back to the material before drum drying. As shown in table 1, xanthophyll stability increased as the concentration of water solubles increased when the samples were not treated with ethoxyquin. Ethoxyquin increased xanthophyll stability, regardless of amount of water solubles present. In fact, ethoxyquin stabilized xanthophyll more than any level of water solubles. There appeared to be a slight synergistic effect between the water solubles and ethoxyquin. This is especially seen in the 12-week data. These data show that some water solubles are necessary for ethoxyquin to provide maximum protection to the xanthophyll, but it looks as though 12-1/2 percent or less would be enough.

Table 1.--Effect of water solubles on xanthophyll stability in drum-dried PRO-XAN[1]

Solubles added, percent	Percent of xanthophyll retained			
	4 Weeks		12 weeks	
	Untreated	Treated[2]	Untreated	Treated
0 - - - - - - - - - -	57	76	28	51
12-1/2 - - - - - - -	59	79	32	59
25[3] - - - - - - - - -	62	77	38	60
37-1/2 - - - - - - -	60	72	38	56

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[1] Samples dried to 11-13 percent moisture, stored in open containers at 100°F.

[2] Samples treated with 0.125 percent ethoxyquin.

[3] Equivalent to normally prepared PRO-XAN.

Different batches of untreated PRO-XAN have different xanthophyll stability (table 2). This might be due to differences in the amount

Table 2.--Xanthophyll stability in different preparations of drum-dried PRO-XAN at 100° F.[1]

Sample[2]	Initial xanthophyll, mg./lb.	Percent of xanthophyll retained			
		4 weeks		12 weeks	
		Untreated	Treated[3]	Untreated	Treated
Preparation 1	473	70	79	46	69
	482	69	78	47	68
	491	69	77	48	68
Preparation 2	275	56	82	27	56
	286	60	83	27	56
	296	56	79	28	58

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[1] Stored in open containers.

[2] Samples prepared from unwashed concentrate.

[3] Samples treated with 0.125 percent ethoxyquin.

of natural antioxidants removed or destroyed during processing, or to differences in the xanthophyll content of the product. Another possible explanation might be the formation of "pro-oxidants," that is, peroxides, during drying which result from fatty acid oxidation. Whatever the reason, however, ethoxyquin affords a similar degree of protection, an average of 68 percent xanthophyll retention in preparation 1 and 58 percent in preparation 2 after 12 weeks' storage. This level of xanthophyll retention is similar to that reported for ethoxyquin treated dehy (3). Notice also that xanthophyll retention is the same at 12 weeks for ethoxyquin-treated PRO-XAN as it was at 4 weeks for unprotected PRO-XAN. This further emphasizes the importance of antioxidant treatment for the drum-dried product.

Various oils and fats are quite effective in reducing carotene loss in dehy (2). Also, small amounts of fat or oil are frequently added to dehy during the pelleting operation to decrease dust problems. During short term storage, oil, especially when combined with ethoxyquin, caused a small increase in xanthophyll stability in PRO-XAN (Table 3).

Table 3.--Effect of oil and ethoxyquin on xanthophyll stability in drum-dried PRO-XAN stored at 100° F.[1]

Oil added, percent	Percent of xanthophyll retained			
	4 weeks		12 weeks	
	Untreated	Treated[2]	Untreated	Treated
0 - - - - - - -	56	82	28	56
5 - - - - - - -	59	84	29	57
10 - - - - - - -	60	88	27	53
20 - - - - - - -	58	88	23	49

[1] Initial xanthophyll content = 287 mg./lb. Samples stored in open containers.

[2] Samples treated with 0.125 percent ethoxyquin.

After 12 weeks, however, more than 5 percent oil tended to decrease xanthophyll stability. This is probably due to the buildup of pro-oxidants from the breakdown of the oil itself.

Even though our main concern was xanthophyll stability, studies were also carried out on carotene stability. In recent years, carotene in alfalfa has not been as important as xanthophyll, because of the low cost of synthetic vitamin A. Nonetheless, it is present in PRO-XAN at about one-half the concentration of xanthophyll and, therefore, its stability is worth considering. All of the variables discussed in this paper have a more pronounced effect on carotene storage stability than on xanthophyll stability. However, oil, whether alone or in combination with ethoxyquin, has the greatest effect on carotene stability (table 4).

Table 4.--Effect of oil and ethoxyquin on carotene stability in drum-dried PRO-XAN stored at 100° F.[1]

Oil added, percent	Percent of carotene retained			
	4 weeks		12 weeks	
	Untreated	Treated[2]	Untreated	Treated
0	40	73	13	44
5	53	85	21	46
10	64	91	28	59
20	70	97	28	59

[1] Initial carotene content = 212 mg./lb. Samples stored in open containers.

[2] Samples treated with 0.125 percent ethoxyquin.

In contrast to its effect on xanthophyll, oil has a positive effect at all levels, regardless of the length of storage. An additional advantage of oil is that it improves the color of the product, makes it a more intense green. At this time, it is felt that oil should be added to PRO-XAN, but less than 5 percent. Higher levels not only seem to have an adverse effect on xanthophyll stability but also cause the product to lose its friability.

Product moisture level is one means of measuring drying conditions. It reflects drying temperature as well as the length of time the product is held at temperature. We aim for about 10 percent moisture in dried PRO-XAN. This moisture level gives a free-flowing product which stores well as far as microbial spoilage goes. As shown in table 5, the differences in drum-drying conditions necessary to obtain moisture levels ranging from 7 to 13 percent has little effect on the subsequent storage stability of the xanthophyll. This is similar to the effect of dehydration when alfalfa is dried to this moisture range (3).

Storage temperature has a more pronounced effect on xanthophyll stability than any of the previous variables. The storage life of PRO-XAN can be increased fourfold just by storing the product at 36°F. instead of 100° (fig. 2). Half of the xanthophyll was lost after

Table 5.--Xanthophyll stability in PRO-XAN drum dried to different moisture contents and stored at 100° F.[1]

Sample[2] moisture, percent	Percent of xanthophyll retained			
	4 weeks		12 weeks	
	Untreated	Treated[3]	Untreated	Treated
7	69	79	42	68
9	70	79	46	69
11	69	78	47	68
13	70	77	48	68

[1] Stored in open containers.

[2] Samples prepared from unwashed concentrate.

[3] Samples treated with 0.125 percent ethoxyquin.

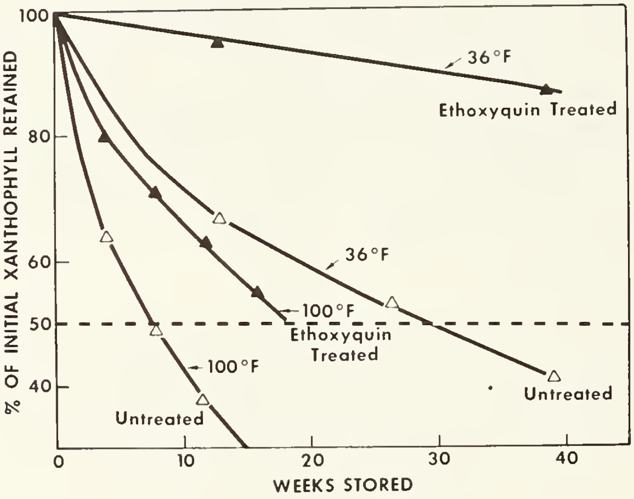

Figure 2.--Effect of temperature and ethoxyquin on xanthophyll stability in drum dried PRO-XAN during open storage.

7-1/2 weeks at 100°. This period increased to 29 weeks when the storage temperature was lowered to 36°. With ethoxyquin treatment, at the colder temperature xanthophyll stability was extremely good, over 85 percent retention even after 39 weeks storage.

The best means of reducing xanthophyll loss investigated was inert atmosphere storage. Samples of PRO-XAN stored in sealed vials with no access to air lost virtually no xanthophyll, even after 6 months at 100° F. Inert gas storage is the method choice for preserving carotenoids in dehydrated forages prior to marketing, and we are sure it will also be for PRO-XAN. However, under conditions where inert gas storage is not practical, it would be worth considering cold storage. This might be feasible since PRO-XAN is a low-bulk, high-density product.

Now that I have covered the six factors that I set out to cover, I would like to briefly discuss the stability of the commercially prepared PRO-XAN (table 6).

Table 6.--Xanthophyll stability in air-dried PRO-XAN, drum-dried PRO-XAN, and dehy[1] stored at 100° F. for 4 weeks

Sample	Initial xanthophyll, mg./lb.	Percent of xanthophyll retained	
		Untreated	Treated[2]
Air dried PRO-XAN	440	78	--
	317	84	90
	396	86	96
Drum-dried PRO-XAN	473	70	79
	294	56	79
Dehy	178	56	79
	171	61	85
	138	60	86

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[1] Stored in open containers.

[2] Samples treated with 0.125 percent ethoxyquin.

Drum-dried PRO-XAN is a thin crumbly sheet that powders readily. Rotary-air-dried PRO-XAN forms hard granules. We are presently carrying out a study on the xanthophyll stability of the air-dried material. Four weeks' storage data are shown in table 6. The rotary air-dried product, even without ethoxyquin treatment, retained considerably more xanthophyll than either drum-dried PRO-XAN or dehydrated alfalfa meal. The improvement in carotene stability is even more dramatic. The rotary air-dried PRO-XAN retained twice as much carotene as did dehy. This surprising degree of carotene stability puts PRO-XAN into the running as a stable source of vitamin A activity. Further research is needed to define the effects of process variables on carotenoid stability and to determine PRO-XAN's stability in mixed feeds, both before and after pelleting. It is hoped that the results of this research will decrease the need for synthetic vitamin A products in rations where alfalfa products are already used for their xanthophyll contribution.

Summary

We have found that the same variables that affect xanthophyll stability during the storage of dehy, affect xanthophyll stability during the storage of drum-dried PRO-XAN, and that they do so to the same extent. Antioxidant protection during storage is a necessity for PRO-XAN, just as it is for dehy. Treatment of the dried product with a small amount of oil is recommended, but definitely less than 5 percent. This will help decrease dust problems when PRO-XAN is ground in addition to offering a degree of protection to both carotene and xanthophyll. Wherever possible, inert gas storage should be employed. It is by far the most effective means of retarding xanthophyll breakdown known. Lastly, PRO-XAN, dried in a conventional hot-air rotary drier, is considerably more stable than dehy. This combined with its negligible fiber content should make it a more reliable source of both carotene and xanthophyll for poultry than conventional pigmentation sources.

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SPECIALLY PREPARED ALFALFA MEALS FOR BABY PIGS

E. R. Peo, Jr., B. Moser, and P. J. Cunningham,
Department of Animal Science, University of Nebr.
and

G. O. Kohler and A. L. Livingston
Western Marketing and Nutrition Research Division, Agricultural
Research Service, USDA, Berkeley, Calif.

One hundred and sixty early-weaned baby pigs averaging 9.7 lb. of body weight were used as a biological tool in two experiments to determine the effect of drying method and saponin content on the potential nutritive value of alfalfa meal. Average daily gain, feed intake, and feed conversion of the pigs were used as criteria of response.

In the first experiment, alfalfa meals dried at 250°, 270°, and 290° F. or freeze-dried and averaging about 25 percent crude protein were used to replace one-half of the supplemental protein in a 16 percent protein corn-soybean meal basal diet. Diets containing the alfalfa meals calculated about 3.5 percent more crude fiber than the corn-soy basal diet. Calcium and phosphorus levels were held constant across all dietary treatments, but vitamin levels exceeded the pigs' requirement in the diets containing the alfalfa meals.

Average daily gain, feed intake, and gain/feed ratio (lb.) were 0.49, 1.05, 0.48; 0.42, 1.05, 0.40; 0.44, 1.24, 0.42; 0.40, 1.02, 0.40; and 0.44, 0.85, and 0.52, respectively, for the corn-soy basal, 250°, 270°, 290°F., and freeze-dried alfalfa meal diets. Pigs fed the corn-soy basal gained significantly faster ($P < .01$) than those fed the alfalfa diets. The most pronounced observation was the highly significant ($P < 0.005$) improvement in the gain/feed ratio of pigs fed the freeze-dried alfalfa meal diet, suggesting that nutrient availability or utilization was markedly enhanced by the freeze-dried processing method. This observation was consistent and amounted to a 30 percent improvement in feed conversion for pigs fed the freeze-dried meal when compared with those fed the heat-dried meals.

In the second experiment, alfalfa meals selected for high and low saponin content were evaluated. Average daily gain, feed intake and gain/feed ratio (lb.) were 0.45, 0.96, 0.47; 0.32, 0.89, 0.35; 0.28, 0.78, 0.36; 0.34, 0.73, 0.47; and 0.34, 0.79, 0.44 for pigs fed the corn-soy basal and MOAPA, 69-7 (low saponin), MIL (low saponin), and MIH (high saponin) alfalfa meal diets, respectively. Pigs fed the corn-soy basal diet gained more rapidly and more efficiently than those fed the alfalfa meal diets. In this study, the corn-soy basal contained the same crude fiber content as the alfalfa meal diets. Pigs fed MOAPA consumed significantly more feed, but were significantly less efficient in feed conversion than those fed the other alfalfa meals. Pigs fed MIL gained significantly faster and more efficiently than those fed 69-7. The differences in performance of pigs fed high versus low saponin alfalfa were not significant.

UNIDENTIFIED GROWTH AND REPRODUCTION FACTORS IN ALFALFA

G. M. Briggs, K. McNutt, Department of Nutritional Sciences,
University of California, Berkeley, Calif.

and

G. O. Kohler[1], Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA, Berkeley, Calif.

Animal nutrition is well on its way to becoming an exact science. Most feeds manufactured today are formulated by computer to supply the known requirements of a specific type of animal by use of available ingredients in the least cost combination. This technique is based upon the assumption that all of the animal's requirements are known on a quantitative basis and that the exact amounts of biologically available essential minerals, vitamins, amino acids, and energy in each feed ingredient are known. While the nutritionist uses the computer to formulate practical feeds, he always has a question in the back of his head. Do the ingredients the computer selects to supply the known requirements also supply adequate amounts of unidentified factors necessary for optimum performance--be it growth rate, feed efficiency, reproduction, or milk production. Such unidentified growth factors (UGF) have been reported to be present in whey, dehydrated alfalfa, fish solubles, and distillers solubles and other byproducts from fermentation industries. There is evidence that these various products are not all supplying the same sort of activity. Hence, we must speak of UGF's rather than UGF.

It is not easy to consistently demonstrate UGF effects in animals fed practical type rations. Sometimes a significant response is obtained upon addition of UGF source while at other times, no response is obtained even though the basal ration is presumably the same. Among the explanations proposed for these discrepancies are (1) that the feed ingredients used in the basal ration are variable in UGF content; (2) that body stores in the young animals vary due to differences in maternal diets; and (3) that variable stress factors such as bacterial infections and variations in intestinal microflora might be involved. However, as the result of variability in response some nutritionists have become "nonbelievers" in UGF. Others, who are "believers" such as I am or who just don't want to take a chance on obtaining suboptimal responses part of the time, program minimum amounts of UGF sources in animal rations, and especially in rations designed for breeding stock, egg production, or other purposes where additional performance and nutritional stress is involved. It is interesting to note that the same type of division of opinion developed after the isolation and characterization of folic acid. Some scientists were bold enough to state that this was the last of the water-soluble UGF factors which would be found since animals could be grown on purified rations supplemented with all known vitamins at rates comparable to those obtained on practical rations. Yet several years later vitamin B_{12} was discovered and is now known to be of practical significance. Vitamin B_{12} was isolated by the use of a microbiological assay.

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[1] Presented by G. O. Kohler.

Nonetheless the burden of proof lies with the affirmative, so work continues on UGF at a number of laboratories around the world. We feel that aside from the importance from a basic knowledge viewpoint, it is important to the alfalfa-processing industry that alfalfa UGF be studied so that all of the values of alfalfa can be effectively utilized in modern computer-controlled feed formulation.

Accordingly we have set up a cooperative effort between the WRRL and George Briggs at the University of California, Berkeley. At this stage of the work, it is essential to have the most reproducible assay attainable regardless of whether the organism or animal used is of practical importance or not. Progress has not been rapid since the problem is a difficult one and is being carried along by graduate students and with a low level of input from our staff.

Table 1 shows the results of an experiment using hamsters fed on a commercial laboratory animal ration, a purified test basal ration, and the latter supplemented with 10 percent alfalfa. It will be noted that the alfalfa group pulled ahead of the controls in the first and second weeks of the test. However, as time went on the control groups began to catch up with the alfalfa group until at 5 weeks there was little difference between lots. Table 2 shows that mice reacted very similarly. The relatively large early differences were largely wiped out by the end of the experiment, although the alfalfa group was still a little in the lead. In an experiment using white rats there was no real difference between the groups at any time.

Table 3 shows that when guinea pigs were used as the test animal, the differences were greater than with mice and hamsters. The data here are pooled from 8 or 9 experiments and show that the purified diet produced poorer growth than the practical diet (chow) and that adding 10 percent alfalfa meal caused a large growth improvement to bring the animals almost up to the chow-diet levels.

Table 1.--Weight gain of neonatal hamsters fed chow, basal, or alfalfa diets for 5 weeks

Diet	Number of animals per group	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks
Chow	6	17	20	46	58	70
Basal	6	18	18	44	61	73
Alfalfa	6	20	36	53	65	75

Table 2.--Weight gain of mice fed chow, basal, or alfalfa diets for 5 weeks

Diet	Number of animals per group	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks
Chow	8	4.9	7.9	12.8	14.3	14.1
Basal	8	3.1	5.4	12.8	14.4	16.7
Alfalfa	8	6.9	12.1	16.5	18.6	18.8

Table 3.--Weight and weight gain of neonatal guinea pigs after 2 and 3 weeks on diets

Time on diet and item	Chow	Basal	Alfalfa
Two weeks:[1]			
Weight (grams)	218	172	208
Weight gain (grams)	98	52	88
Three weeks:[2]			
Weight (grams)	271	210	254
Weight gain (grams)	151	90	134

[1] Average of 9 studies.

[2] Average of 8 studies.

During the course of the past 2 years a great deal of testing has been done to show unequivocally that various changes in the basal diet could not account for the alfalfa effect. Thus, varying the levels of known vitamins, essential minerals, and essential amino acids, changing the type of carbohydrate used, and adding different levels of purified cellulose did not affect the alfalfa response significantly. The test basal finally adopted is shown in table 4. Thus we have shown that alfalfa meal does

Table 4.--Basal diet

Ingredient	G./kg. diet
Vitamin-free casein	300
Arginine	3
Mineral mix, UC-B	75
Corn oil	30
Water-soluble vitamin mix	20
Fat-soluble vitamin mix	10
Ascorbic acid	2
Choline chloride	2
Cornstarch	200
Cerelose	160
Cellophane spangles	25
Sucrose	173

contain a UGF (s) for guinea pigs and we do have a reasonable assay for it. When I say reasonable, I do not mean we can quantitate UGF in units suitable for computer use. The errors are large, as with all animal assays, and guinea pigs are highly susceptible to colds and other diseases so that necessity of repetition of assays is to be expected. We do feel that the assay would show whether we have destroyed or lost half of the activity in a process. Table 5 gives some indication of the type of graded response obtained when different levels of alfalfa are used. We have done some fractionation and stability work and are continually gaining knowledge as to how to handle samples so as to get more consistent results.

Table 5.--Effects of feeding increasing amounts of alfalfa

Diet	Gain over basal diet	Number of studies
	Grams	
5 Percent alfalfa	29	2
10 Percent alfalfa	32	3
20 Percent alfalfa	49	3
30 Percent alfalfa	76	2
Chow	51	3

The UGF is not extractable with hexane or other nonpolar solvents. Some activity is extracted using alcohol and other polar solvents. It is stable to hot sodium hydroxide treatments.

During the coming year we plan to carry out further studies on the nature of the alfalfa UGF and to determine the effects of wet processing and dehydration variables on the activity. The objective here, of course, is to find how, if possible, to manufacture alfalfa products with more UGF activity. Also on the agenda is a project, which the ADA is sponsoring, which is to test a series of commercial dehydrated alfalfas to determine the degree of uniformity of the product as purchased by the feed manufacturer.

In closing this presentation let me say once more that this area is difficult and progress will continue to be slow. But in the long range it could well be one of the most important projects we have undertaken.

THE EFFECT OF PARTICLE SIZE OF ROUGHAGES IN FINISHING RATIONS FOR STEERS

J. D. Aughtry
Feed Consultant, Indian Wells, Calif.

The beef cattle feedlot industry has been aware for some time that the fineness of grind of roughage is a factor in the performance and management of cattle fattened on high-energy, minimum-roughage rations under modern, commercial feedlot conditions. Only recently has an effort been made to describe fineness of grind by measuring the particle size distribution of roughage processed for incorporation into beef-cattle finishing rations and to estimate the effect of particle-size distribution on performance of fattening cattle. Feeding trials related to this problem were conducted at the Shepard Feedlot, Hale Center, Texas, during 1969 and 1970 for the American Dehydrators Association.

Objective

The objective of these trials was to evaluate the physical condition of several alfalfa products as it relates to fattening steers for slaughter under feedlot conditions. One part of the investigation was to describe the physical condition of alfalfa in terms of particle-size distribution as determined by mechanical analysis in the laboratory and utilize such information in the development of new alfalfa products better adapted to feedlot use. More specifically, the objective was to compare regular dehydrated alfalfa pellets and a newly developed pelleted alfalfa product with cubed alfalfa as the only source of roughage in high-energy, minimum-roughage feedlot rations. By design, roughage factor was placed under maximum pressure in computerized feed formulas which differed only in regard to the physical condition of the three alfalfa products used.

Experimental

1969 Trials

Feeding trials were conducted in 1969 to compare three alfalfa products in three replications under feedlot conditions when formulated at equal levels into the same high-energy, minimum-roughage feed for fattening cattle. The three alfalfa products differing in physical condition and constituting the three treatments of the experiment were: (A) Alfalfa cubes, sun-cured, (B) coarsely ground dehy in 3/8-inch pellets, and (C) regular 1/4-inch dehy pellets. The particle-size distribution of the three products was:

Length	A	B	C
Fines - - - - - - - - - - -	13.40	32.78	65.41
Fines - 1/8 inch - - - -	19.49	28.80	34.59
1/8 - 1/2 inch - - - - -	45.46	38.42	--
1/2 - 1 inch - - - - - - -	12.95	--	--
Over 1 inch - - - - - - -	8.68	--	--

The three feed formulas compared appear in table 1. Treatment A was the regular feeding program at the Shepard Feedlot and served as the "control" for the experiment. Products B and C, constituting treatments B and C, were made from alfalfa harvested from the same field and were considered identical except for size of pellet and fineness of grind.

It is apparent from the figures above that the special B pellets were coarser than the conventional dehy pellets but were far from comparable in that regard to the John Deere cubes in treatment A.

Three hundred and sixty head of #1 Oakie Steers, weighing an average of 760 pounds, were selected from a larger group of cattle and sorted into nine uniform pens of 40 head each. At the time of sorting the cattle had been in the feedlot for about 30 days and were on full feed. All cattle were brought up to the top feed on the regular program at the feedlot which involved both alfalfa cubes and cottonseed hulls as roughage sources. After sorting and weighing, the cattle were placed in their respective pens and put on treatment. After 1 week on treatment, all pens were reweighed and the experiment began. Feed and water were not withheld prior to weighing at the end of the trial. Cattle were weighed early in the morning with a 4 percent shrink - the standard basis for selling cattle in the area.

1970 Trials

Except for the three alfalfa products tested, the 1970 trials were essentially a duplication of those conducted a year earlier. All three alfalfa products came from the same field and cubes were blended in a 1:3 ratio with the 3/8-inch and 1/4-inch pellets to increase the average particle length of the B and C treatments. The resulting treatments were: (A) Alfalfa dehy in John Deere cubes, (B) 75 percent coarsely ground dehy in 3/8-inch pellets

+ 25 percent cubes, and (C) 75 percent dehy in 1/4-inch pellets + 25 percent cubes. The particle size distribution of each treatment was:

	Alfalfa dehy products		
Length	John Deere cubes	Coarse 3/8-inch pellets	1/4-inch pellets
Fines - - - - - -	32.89	42.02	51.70
Fines - 1/8 inch -	13.86	14.92	16.13
1/8 - 1/2 inch - -	24.60	24.14	25.00
1/2 - 1 inch - - -	28.65	18.52	7.17
Over 1 inch - -	--	--	--

In comparison to the treatments compared in 1969, there was less "roughness" in treatment A, about the same in treatment B, and considerably more in treatment C.

The three feed formulas compared in 1970 appear in table 2.

Table 1.--Percentage composition of experimental feed formulas - 1969 trials

	Treatment		
Ingredients	A	B	C
Grain (wheat and milo) - - - -	71.50	71.50	71.50
Molasses, cane - - - - - - - - -	2.50	2.50	2.50
Fat - - - - - - - - - - - - - - - - -	3.00	3.00	3.00
Alfalfa cubes - - - - - - - - -	18.00	--	--
Alfalfa dehy special pellets	--	18.00	--
Alfalfa dehy regular pellets	--	--	18.00
Premix - - - - - - - - - - - - - -	5.00	5.00	5.00
Total	100.00	100.00	100.00

Table 2.--Percentage composition of experimental feed formulas - 1970 trials

	Treatment		
Ingredients	A	B	C
Grain (wheat and milo) - - - -	70.00	70.00	70.00
Molasses - - - - - - - - - - - - -	2.50	2.50	2.50
Fat - - - - - - - - - - - - - - - - -	3.25	3.25	3.25
Alfalfa cubes - - - - - - - - - -	20.00	5.00	5.00
Alfalfa pellets - "B" - - - -	--	15.00	--
Alfalfa pellets - "C" - - - -	--	--	15.00
Premix - - - - - - - - - - - - -	4.25	4.25	4.25
Total	100.00	100.00	100.00

Results

The results of the 1969 trial appear in table 3. Over the 119-day feeding period, the average daily gain and feed conversion for treatments A, B and C, respectively, were 2.71, 8.10; 2.66, 8.27; and 2.55, 8.62. Feed consumption expressed as percent average live weight in the same order was 2.42, 2.49, and 2.63. With all alfalfa products charged at the same price, the cost of gain was $22.36, $22.98 and $23.80, respectively. No significant differences were observed in dressing percentage, quality grade, or yield grade among the three treatments. More abscessed livers and distinctly more keratinization of rumen walls were observed in cattle fed the regular dehy pellets. The cattle fed the cubes were normal and the special pellets were intermediate in this regard. All of these data and observations suggest that the special pellets had more roughage factor than the regular dehy pellets but less than the cubes, and that the differences were sufficient to affect the performance.

The figures in table 4 reflect the results of the 1970 trials. The average daily gain and feed conversion for the 140-day period for treatments A, B, and C, respectively, were 2.84, 8.22; 2.75, 8.36; and 2.68, 8.49. Feed consumption expressed as percent of average live weight in the same order was 2.36, 2.49, and 2.55. With all alfalfa products charged at the same price, the cost of gain was $23.32, $23.61, and $24.02, respectively. Carcass data revealed no significant differences in dressing percentage, quality grade, or yield grade. Unfortunately, observations on liver and tripe condemnations were not properly recorded. It is apparent that the data from the 1970 trials are very similar and follow the same trends as those of 1969.

Discussion

The three feed formulas compared in each of the two Shepard trials were formulated with a computer. In each trial the three formulas fed were equal in estimated net energy, dry matter, protein, fat, fiber, nitrogen-free extract, ash, calcium, phosphorous, vitamins, hormones, and antibiotics. In 1969, the A treatment was the standard feeding program of the feedlot during the course of the trial. The A treatment in 1970 differed from the standard program only in that dehy cubes were substituted for the sun-cured cubes in regular use at the feedlot at the time. In both cases, then, the control ration was a normal, practical feedlot formula.

A classical statistical analysis is seldom applicable to field trials such as those under discussion here. The size and design of the experiments precludes a small number of degrees of

Table 3.--Feedlot performance of three alfalfa products at Shepard feedlot, 1969

Item	Alfalfa product Alfalfa cubes (A)	 Special 3/8-inch dehy pellets (B)	 1/4-inch dehy pellets (C)
Number of head	120	120	120
Days on feed	119	119	119
Initial weight - lb.	761	766	752
Final weight - lb[1]	1,084	1,083	1,055
Gain head	323	317	303
Average daily gain	2.71	2.66	2.55
Feed consumption	21.98	21.97	21.95
Lb. feed/lb. gain	8.10	8.27	8.62
Cost/lb. gain[2]	22.36	22.98	23.80
Head slaughtered	119	114	117
Dressing percentage	63.8	63.9	63.9
Quality grade[3]	11.3	11.5	11.1
Yield grade	2.82	2.80	2.79
Condemned livers, percent	47.2	47.9	55.7
Condemned tripe, percent	21.8	28.3	29.1

[1] Weighed with 4 percent shrink. No withdrawal of feed or water.

[2] All alfalfa products charged at same price.

[3] Good = 9-11; Choice = 12-14; Prime = 15-17.

freedom and, consequently, larger differences for statistical significance. One must rely on replications, trends, and consistency of data for interpretation of results.

The objective of this investigation was to compare several alfalfa products differing only in physical condition when formulated at equal levels into standard, practical feedlot rations and fed to fattening steers under feedlot conditions. These trials were not designed to gather any information whatever on the comparative value of sun-cured and dehydrated alfalfa. Neither were they designed to deal in any way with the level of roughage, energy, or any particular ingredient in feedlot rations.

Alfalfa in some form appears in almost all feedlot rations. It may enter at a high level to supply, among other things, Table 4.--Feedlot performance of three alfalfa products at Shepard feedlot, 1970

Item	Alfalfa cubes (A)	Special 3/8-inch dehy pellets (B)	1/4-inch dehy pellets (C)
Number of head	120	120	120
Days on feed	140	140	140
Initial weight lb.	767	753	749
Final weight lb.[1]	1,168	1,147	1,133
Gain/head	401	394	384
Average daily gain	2.84	2.75	2.68
Feed consumption	23.42	23.02	22.78
Lb. feed/lb. gain	8.22	8.36	8.49
Cost/lb gain[2]	23.32	23.61	24.02
Head slaughtered	116	118	119
Dressing percentage	62.2	61.7	62.2
Quality grade[3]	12.1	12.2	12.2
Yield grade	2.75	3.20	3.10

[1] Weighed with 4 percent shrink. No withdrawal of feed or water.

[2] All alfalfa products charged at same price.

[3] Good = 9-11; Choice = 12-14; Prime = 15-17.

roughage factor or it may enter at low levels on a supplementary basis. Should the dehy industry find it advantageous to go for the former it is imperative that the product be ground coarsely enough to preserve roughage factor. On the other hand, where adequate roughage factor is supplied by some other ingredient such as corn silage, particle-size distribution is of little if any significance.

It became apparent early in this study that some pellet mills are inefficient but effective grinders. The short, chopped alfalfa is a superior product as it leaves the dehydrator, but, regardless of whether it goes through the hammer mill or directly to the pellet mill, the particle-size distribution of the final product is below the target specification set for new dehy products.

The results of the survey indicate that if the alfalfa dehy industry could manufacture alfalfa pellets whose particle-size distribution falls within the ranges reported above, it would in effect preserve as much of the roughage factor inherent to alfalfa as such competitive products as ground, baled hay and

John Deere cubes. The latter two products have been and still are the principal competitive products for dehy pellets in western feedlots.

Roughage factor in ruminant feeds is a very complex nutritional entity. Concentrate: roughage ratios do not adequately characterize roughage factor in modern ruminant rations. Some ingredients classified as concentrates contribute roughage factor to feed formulas. It is reasonable to assume that significant interactions exist between fiber level, source of fiber, and physical condition as it relates to particle-size distribution. Other factors, such as volume weight, may also be involved. The experimental technique employed in replicated field trials, such as the Shepard trials, are inadequate to fully clarify the subject. Such trials can, however, demonstrate that performance in the feedlot can be conditioned by the fineness with which alfalfa is ground for the rations tested. Further research, under carefully controlled conditions, can clarify the fundamentals involved.

The feedlot industry is operating in the computer age of feed formulation. The specification of feed formulas can be conditioned by many controls and restrictions, all of which require specific mathematical coefficients. Coefficients reflecting the roughage factor of all ingredients used in ruminant feeds would be a useful tool in formulating commercial feedlot rations. Researchers could contribute much in this area by a greater use of computer techniques in formulating experimental feeds for ruminants.

Summary

The Shepard trials over the 2-year period may be summarized as follows:

1. The data from the 1969 and 1970 trials are similar, consistent, and reflect similar trends in performance by the several alfalfa products tested with steers fed under feedlot conditions.

2. The trend in performance in both trials in terms of average daily gain and feed conversion plus the pattern and degree keratinization of rumen walls in the 1969 trials is strong evidence that the fineness of grind of the alfalfa products tested exerted an appreciable effect on the feedlot performance of the rations tested.

3. The degree and pattern of keratinization of rumen walls in the 1969 trial strongly suggests that roughage factor was involved and associated with fineness of grind. Keratinization

was extensive with the finely ground, pelleted alfalfa while the cubed product produced none at all. The coarse pellets were intermediate in this regard.

4. The difference in performance between the cubed alfalfa in 3/8-inch pellets was very small but probably real. Such pellets are acceptable to feedlots and several such products are already commonly used. The dehy industry should, however, make further progress in this area by increasing the effective particle length of pelleted products produced for feedlots.

5. Additional research is obviously necessary to clarify the fundamental principles related to the observations reported from the Shepard trials.

ALFALFA PRODUCTS IN RATIONS FOR CATTLE

Terry Klopfenstein
University of Nebraska, Lincoln

Dehydrated alfalfa products are used in many different types of cattle rations. The value of dehy in any one of these rations depends to a large extent upon the purpose for which the dehy is fed. For calves on growing programs, protein, vitamins, minerals, and energy content of the dehy are all very important. In cattle finishing rations, roughage characteristics of the dehy and the protein, vitamin, and mineral content are very important. However, the contribution of energy from the dehy may be relatively unimportant in the finishing ration. It is important, then, to make the best possible advantage of dehy in any ration. Three feeding trials have been conducted which demonstrate the above points.

In the first of these trials, four dehydrated alfalfa products were fed to growing steers which weighed approximately 500 lb. These cattle were fed the four alfalfa products and were supplied with salt and water free-choice. The products were second-cutting whole plant and stems made from the same field at the same time and third-cutting stems and whole plant made from the same field at the same time. The third cutting was made at an earlier stage of plant maturity than the second cutting. Four qualities of dehy products were therefore obtained. Protein contents for the second-cutting stems, second-cutting whole plant, third-cutting stems, and third-cutting whole plant, respectively, were 14.9, 16.1, 14.4, and 16.8. Complete separation of leaves from stems apparently was not accomplished in this case. Results of this feeding trial are shown in table 1.

Table 1.--Growing steer performance[1]

	2nd cutting		3rd cutting	
Item	Stems	Whole plant	Stems	Whole plant
Number of animals - - -	14	14	14	14
Initial weight, lb. - -	506	499	508	506
Daily gain, lb. - - - -	1.63	1.78	1.85	1.91
Feed/gain - - - - - - -	13.0	12.6	12.0	11.9

[1] Length of trial, 113 days.

The cattle gained from 1.6 to 1.9 lb. per day depending on the quality of the alfalfa product. Pounds of feed required per pound of gain ranged from 12 to 13. There was less reduction in gain due to removal of the leaves than was expected. This would indicate that the alfalfa stems are an excellent energy source for growing cattle, assuming they are harvested at a reasonable stage of maturity. These data also indicate that the alfalfa products can comprise a complete ration for growing programs for cattle.

The second trial which was conducted involved performance of growing lambs. These lambs were fed pelleted 17 percent protein dehy or a ration composed of 70 percent corn and 30 percent dehy or a ration composed of 50 percent dehy and 50 percent ground wheat straw. These rations were formulated by balancing for protein between the energy source (corn or straw) and dehy. Table 2 shows the results of this performance trial.

Table 2.--Growing lamb performance[1]

Item	Dehy	Dehy plus corn[2]	Dehy plus straw[3]
Number of animals	8	8	8
Daily feed, lb.	3.80	2.45	1.73
Daily gain, lb.	.38	.35	.03
Feed/gain	10.0	7.0	---

[1] 50-day test.

[2] 70 percent corn.

[3] 50 percent straw.

The dehy plus corn ration was limit-fed in order to produce gains approximately equal to that obtained on the dehy ration. As can be seen, lambs fed dehy plus corn required only 70 percent as much feed per pound of gain as did the lambs fed dehy alone. Lambs fed dehy plus straw just maintained their weight. These data indicate that energy is the first limiting nutrient in dehydrated alfalfa for growing ruminants. Lambs fed dehy with corn gained faster than those fed dehy alone, whereas, those fed dehy plus straw gained slower than those fed dehy alone. The practical application of this is to use dehy to supply those nutrients which it contains in sizeable quantities. This would include protein, vitamins, and minerals.

The final trial which was conducted involves the use of dehy in cattle finishing rations. Dehy is certainly a good source of protein, vitamins, and minerals in finishing rations but may not be important as a supplier of energy. Table 3 is a summary of three Nebraska trials which show the effect of roughage in cattle finishing rations. The roughage in this case was one-half chopped alfalfa hay and one-half corncobs or brome hay. This is typical of the effect of roughage as shown by several workers. These data were shown to demonstrate the particular effects of adding roughage to an all concentrate ration. In many cases roughages are compared in finishing rations without the appropriate control which is the all concentrate ration. Addition of roughage to all concentrate rations increases daily gain, increases feed consumption, and reduces incidences of abscessed liver and other digestive disturbances.

Table 3.--Roughage levels in finishing rations[1]

Item	All concentrate	Roughage level[2]	
		5 percent	15 percent
Daily feed, lb - - - - - - - - - - -	20.8	23.0	25.0
Daily gain, lb - - - - - - - - - - -	2.76	3.09	3.07
Grain/100 lb. of gain, lb - - - -	751	712	692
Roughage/100 lb. of gain, lb - - -	---	38	122
Roughage value, percent - - - - -	---	100	48
Abscessed livers, percent - - - -	49	34	24

[1] Summary of 3 Nebraska trials.

[2] Roughage was half chopped hay and half corncobs or brome hay.

With this information in mind a finishing trial was conducted to determine whether normal fine ground dehy pellets supplied roughage factor to a finishing ration and whether coarse 3/8-inch dehy pellets would supply more of this same roughage factor. Two hundred and seventy head of cattle were allotted to 18 pens of 15 head each. Nine rations were fed to two pens of cattle each.

The results of laboratory analysis determined the fiber length of the two alfalfa products as shown in table 4.

Table 4.--Results of analysis

Fiber length	1/4-inch pellets	3/8-inch pellets
	Percent	Percent
Fines	59.29	32.51
Fines - 1/8-inch	16.63	9.72
1/8 - 1/4-inch	8.80	10.14
1/4 - 1/2-inch	15.28	12.59
1/2-inch and over	---	35.04

As can be seen, over 50 percent of the coarse dehy pellets had fiber lengths of more than 1/8-inch. In contrast the fine ground dehy pellet had over 75 percent of the fiber being less than 1/8-inch.

The nine treatments in this trial are shown in table 5. The controls were an all-concentrate ration and rations with 5 percent and 10 percent corn silage. Fine and coarse dehy were both fed at 5, 10, and 15 percent of the ration. As seen in table 5, addition of 10 percent corn silage increased gain compared with the all-concentrate ration. Dry-matter intake was also increased. This is the typical effect of adding roughage to an all-concentrate ration. The efficiency of gain was similar to the

Table 5.--Level and form of dehy in cattle finishing rations

Treatment	Daily gain, lb./day[1]	Feed intake, lb./day	Feed/lb. gain	Percent abscessed livers
All-concentrate	2.64	16.9	6.4	79.3
5 percent corn silage	2.66	18.7	7.1	53.6
10 percent corn silage	3.02	19.6	6.5	33.3
5 percent fine dehy	2.77	18.1	6.6	66.7
10 percent fine dehy	2.97	19.0	6.4	57.1
15 percent fine dehy	3.00	19.2	6.4	75.9
5 percent coarse dehy	2.85	18.5	6.5	50.0
10 percent coarse dehy	2.80	19.4	6.9	75.0
15 percent coarse dehy	2.98	20.4	6.9	76.7

[1] Gain adjusted to equal dressing percentage.

all-concentrate ration and abscessed livers were decreased considerably. The 10 and 15 percent levels of fine dehy also increased average daily gain similar to the increase obtained with corn silage. Intake was also increased and feed efficiency was as good as the all-concentrate ration. This would indicate that the fine-ground dehy at these levels was exhibiting a roughage effect. Abscessed livers were not decreased to the extent obtained with the 10 percent corn silage ration.

This is an indication that the fine-ground dehy was not acting completely as a roughage. The 5 percent coarse dehy tended to increase daily gain and feed intake compared with the all-concentrate ration. In addition, efficiency of gain was similar and the incidence of abscessed livers was decreased considerably. However, additions of 10 and 15 percent coarse dehy, although they stimulated average daily gain and feed intake compared with the all-concentrate control, did not improve feed efficiency and degree of abscessed livers. These results were somewhat unexpected but may be explained in view of closer analysis of the data.

Variation in performance of the cattle within the lots on the 10 and 15 percent coarse alfalfa would indicate that the animals were somehow responding differently to the treatment. It was also observed in feeding the cattle that the coarse 3/8-inch dehy pellets tended to separate from the ration as it entered the feed bunk. It was therefore possible for the animals to sort the coarse pellets if they so desired. During the feeding trial it did not appear that the animals were sorting the coarse dehy pellets; however, it is difficult to determine if one animal is eating more or less than his share of the pellets. At the present time, the only plausible explanation for the depressed performance in the 10 or 15 percent coarse dehy products is that the animals were sorting these products and therefore, not getting even consumption of the roughage and concentrate.

Table 6 shows results from a Minnesota trial conducted to show the effect of additions of fine-ground dehy to all-concentrate rations. In this case, as in the Nebraska trial, 5 percent addition of dehy had no important effect on the performance of the cattle except that it lowered efficiency of gain. Addition of 10 and 15 percent dehy increased average daily gain and increased feed intake, again indicating a roughage effect. Efficiency of gain was somewhat lower when the dehy products were fed compared to the all-concentrate ration. The Minnesota data support the Nebraska data which indicate that fine-ground dehy does in fact contain some roughage characteristics. Whether or not this is sufficient for cattle finishing rations is yet to be determined. Future work at Nebraska will try to determine more precisely the value of the coarse-ground products and will include studies where the pellet is crumbled to insure proper inclusion into the ration.

Table 6.--Performance of cattle fed various levels of dehy

Item	Percent dehydrated alfalfa pellets			
	0	5	10	15
Number of steers - - - - - -	39	40	40	39
Average of daily gain, lb. -	2.64	2.64	2.80	2.74
Average of daily feed, lb. -	21.74	22.72	23.94	23.73
Feed/100 lb. gain - - - -	824	862	857	868
Corn replaced by 100 lb. dehy alfalfa, lb.	---	2.3	51	55

Source: University of Minnesota, 1969 Cattle Feeders Day Rpt.

ALFALFA PRODUCTS IN HIGH-UREA RATIONS

W. M. Beeson
Department of Animal Sciences
Purdue University, Lafayette, Ind.

This paper will summarize some of the research that has been conducted on the effect of dehydrated alfalfa meal, alfalfa stem fraction meal, and alfalfa solubles on the utilization of urea by beef cattle.

Dehydrated Alfalfa Meal

Three experiments at Purdue University (5) indicated that a 32 percent natural protein supplement (Supplement A) was superior to a high-urea 64 percent supplement for finishing steers. Steers receiving the 32 percent Supplement A gained significantly more on less feed per unit of gain than steers fed an equivalent amount of protein from a 64 percent high-urea supplement. Daily gains and feed efficiency for the 32 and 64 supplements were, respectively, 2.64 versus 2.37 pounds and 826 versus 860 pounds. The addition of lysine or methionine to the 64 percent supplement caused no significant effect on growth rate or feed conversion. The original 64 percent supplement contained 14 percent of dehydrated alfalfa meal.

In 1964 Beeson et al. (1) formulated the first high-urea supplement which was equal to a natural protein supplement by increasing the dehy from 14 to 51 percent in the formula. Feeding 1.0 pound of the 64 percent supplement daily resulted in an intake of 0.51 pound of dehydrated alfalfa meal. The daily gains and feed efficiency on the Purdue 64 Supplement versus Supplement A (natural protein) were, respectively, 2.22 and 2.32 pounds and 554 and 552 pounds of TDN per 100 pounds of gain. A year later (2) we confirmed these results with steers fed on Supplement A and Purdue 64 that gained 2.56 and 2.55 pounds daily on 543 and 525 pounds of TDN per 100 pounds of gain, respectively. Thus, through the use of dehydrated alfalfa meal, it was possible to formulate a supplement built around urea which would be equal to a natural protein supplement. The formula of the 64 percent supplement is as follows:

Purdue 64 (64 percent protein)

	Pounds
Urea (45 percent N)	200
Cane molasses	140
Dehydrated alfalfa meal (17 percent)	510
Dicalcium phosphate	105
Iodized salt	35
Premix[1]	10
	1000

[1] Premix: 20 million I.U. vitamin A, 10 g. DES, 1250 g. zinc oxide, 4 g. cobalt carbonate, and 7 lb. dehydrated alfalfa meal.

Later Horn and Beeson (6) revealed in carefully controlled balance studies with steers that nitrogen retention was increased 66 percent by feeding 5 percent dehydrated alfalfa meal in a corn and cob meal-urea-mineral diet. In these studies the steers received 227 to 300 grams of dehydrated alfalfa meal daily. This significant improvement in nitrogen utilization from urea by dehydrated alfalfa meal indicates that dehy contains nutritional factors which are essential for the synthesis of bacterial protein from nonprotein nitrogen. Also, in these studies there was a significantly ($P < 0.05$) higher concentration of total ruminal volatile fatty acids in the rumen of steers fed dehy which indicates increased microbial activity. The ruminal fluid of steers fed the basal plus dehy diet contained significantly ($P < 0.01$) higher concentrations of acetic acid than the steers fed the basal diet. Ruminal ammonia blood urea concentrations and digestible energy were not affected by dehy.

Alfalfa Stem Fraction Meal

Using separation milling techniques Chrisman and Kohler (4) separated dehydrated whole plant alfalfa into two fractions. By the use of various air velocities, a dehydrated alfalfa stem fraction meal (DAS) containing 15 percent crude protein and a high protein leaf meal (21 percent protein) were produced. The alfalfa stem fraction meal was used in research with beef cattle at Purdue University.

Van Slyke (8) showed that the addition of DAS to a high-urea semipurified diet resulted in a 19 percent increase in dietary nitrogen retention and a 14 percent increase in absorbed nitrogen retention. No consistent treatment differences were noted in digestible energy, dry-matter digestibility, rumen ammonia, plasma urea, rumen volatile fatty acids, or plasma-free amino acids. When DAS was compared with dehydrated alfalfa meal at a 5 percent level in the diet, no differences in nitrogen retention were observed. These data suggest that dehydrated alfalfa stem fraction meal contains essentially the same factors as dehydrated alfalfa meal for the improvement of protein synthesis from urea.

In feedlot trials we fed steers Purdue 64 supplements containing 52 percent dehy or 52 percent DAS gained at nearly equal rates (2.60 vs. 2.52 pounds daily) (3). The supplements were fed with corn silage and high-moisture ground ear corn. No differences in feed efficiency, loin-eye area, backfat thickness, dressing percentage, or carcass grade were observed.

Klopfenstein et al. (7) found that cattle fed good-quality alfalfa stems ate more feed and gained more rapidly and efficiently than the steers fed poor-quality alfalfa stems or chopped alfalfa hay. In a lamb-feeding trial, a ration of 50 percent good-quality alfalfa stems and 50 percent corn produced results superior to feeding either poor-quality alfalfa stems or chopped alfalfa hay in the ration. These workers concluded that high-quality alfalfa stem fraction meal can be effectively utilized as a source of roughage and other nutrients in cattle and lamb finishing rations.

Alfalfa Solubles

Alfalfa solubles are defined as the solubles that are produced from the pressed juices of alfalfa. The total weight of juice, which can be obtained by a combination of roll and hydraulic pressing, will average about 75 percent of the fresh weight of alfalfa. This juice is then heated and put through a filter system, and a protein coagulum is obtained which contains the carotene and xanthophyll of the juice plus a large proportion of water-soluble protein. The wet protein mass is separated into a chlorophyll-carotene-xanthophyll concentrate and a light-tan protein which is freeze dried to a powder. This powder was dissolved in a 50 percent water solution for these research studies.

Three metabolism trials and two feedlot studies were conducted at Purdue University to determine the effect of adding different levels of alfalfa solubles (AS) on a dry-matter basis to complete mixed diets and liquid supplements. Nitrogen and energy utilization, dry-matter digestibility, ruminal volatile fatty acids, and rumen pH were the criteria used for the metabolism studies, and average daily gain and feed efficiency were the parameter for the feedlot trials.

The basal diet for the metabolism trials was built essentially around ground corn, ground corncobs, urea, minerals, and vitamins. Urea levels in the ration varied from 1.75 to 1.95 percent. The addition of 0.5 percent and 1.0 percent alfalfa solubles on a dry basis to the basal diet indicated a trend toward an improvement in nitrogen utilization from urea, while the addition of 2.0 percent of AS resulted in a slight depression. These differences in nitrogen utilization were not statistically significant. Dry-matter digestibility and digestible energy were significantly improved when 0.5 percent AS was incorporated in the diet.

In two feeding trials, levels of 2.5 percent and 5.0 percent of supplemental alfalfa solubles in a liquid protein supplement were compared with a liquid supplement containing no such source of UGF or with one containing 2.5 percent of distillers solubles.

The liquid supplements were fed either on a 1 pound per head per day basis in the first feeding trial or mixed in a complete mixed ration as 6.25 percent of the total ration in the second feeding trial. In the second experiment, the average daily consumption of liquid supplement amounted to very nearly 1 pound per head daily. So in both trials, the actual daily consumption of alfalfa solubles on the two levels compared amounted to 0.025 and 0.05 pound (11.3 and 22.6 g). In the first feeding trial, the cattle were fed a full-feed of dry rolled corn plus limited corn silage and 1 pound of liquid Purdue 64 supplement. In the second feeding trial, the steers were self-fed on a complete mixed ration composed of 77.70 percent rolled corn, 15.0 percent ground corncobs, 6.25 percent Purdue 64 liquid supplement, 0.70 percent feed-grade limestone, and 0.35 percent salt.

In the two feedlot tests, the addition of either 2.5 percent or 5.0 percent of alfalfa solubles to a high-urea liquid supplement caused no significant improvement in daily gain or feed efficiency over the controls. However, in the first feeding trial, incorporation of 2.5 percent alfalfa solubles in the liquid supplement resulted in a significant ($P < 0.05$) increase in the rate of gain of finishing beef cattle over those fed a liquid supplement containing 2.5 percent distillers solubles or over those whose supplement contained 5.0 percent alfalfa solubles.

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RECYCLING GASES IN THE DEHYDRATOR

G. O. Kohler, J. Chrisman, A. L. Livingston,
R. Edwards, and R. E. Knowles
Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA
Berkeley, Calif.

Recycling stack gases means returning a fraction of the stack gases back to the furnace to replace a part (or all) of the secondary air going into the furnace. Stack gas recycling is an old idea which until recently has been attempted primarily with the objective of recovering the heat from the gases. The economic value of such recovery in relatively small plants such as have been and still are common in the dehydration industry is questionable. If the natural gas cost for producing a ton of dehydrated alfalfa (4) is $3.25 per ton and the savings by recycling is 6.5 percent, the gross savings per ton would be about 20¢. For a 5,000 ton per year production the gross savings would be about $1,000. Considering the capital expenditure for a booster fan (which might be eliminated), for duct work and insulation, and for remodeling of the furnace to accommodate the system and considering also the extra electricity used, the additional maintenance required, and the questions as to whether it would work smoothly, cause fires, etc., gas recycling has gotten nowhere in the alfalfa dehydration industry.

During the past several years, a new element has been injected into the scene which has led to reevaluation of the recycle idea. That element is the militant environmentalist. Whether we like it or not, anti-air pollution laws are already in effect in many States.

Particulate-matter tolerances are set at levels which will be extremely difficult for the industry to meet unless some changes are made. One such possible change which needs investigation is recycling stack gases since both the particulate matter and the odorous components of the part of the effluent put back through the furnace are burned up at that point. One company which has specialized in manufacturing driers for drying sugar beet pulp has developed a recycle system which is claimed to reduce dust loss from about 0.35 percent of the pulp produced to 0.21 percent. While these results were obtained on two commercial plants operated under somewhat different conditions, a 40 to 50 percent reduction is not unreasonable considering the percentage of gas recycled and design of the system. Insofar as I know, no recycle system of this design has been used on this company's plants which operate on alfalfa. We understand that recycle systems are available or are under development by the several other companies which manufacture triple-pass and single-pass drums.

Here at WRRL our initial interest in recycling was generated about 4 years ago as the result of our research on xanthophyll losses during dehydration (3). Prior to this work everyone including ourselves had the idea that since carotene was stable during dehydration, lutein, the main component of alfalfa xanthophyll, would also suffer minor losses. After all, carotene and xanthophylls have identical chemical structures insofar as the carbon backbone is concerned and differ only in the number of hydroxyl groups and the position of a double bond. As so often happens, nature did not conform to our preconceived ideas and we found that very large losses of lutein occurred during the drying process. By these studies, using our pilot-plant Arnold drier, a full-scale Arnold drier, and a Stearns-Roger drier, we were able to show that losses were best correlated with moisture content of the product. Presumably as long as there is enough free moisture remaining in the product, the product will remain at the wet-bulb temperature, which is below 212° F., even though the temperature of the gas around the particles may be held at 250° to 300° F. We also found that the essential amino acids are affected by dehydration in a manner similar to xanthophyll (2).

In considering means of avoiding xanthophyll losses, one approach, obviously, is to develop a control system which is based on rear end product moisture or product temperature rather than dry-bulb gas temperature. We are planning to pursue this line of attack. A second approach is to eliminate oxygen from the dehydrator--that is, to dehydrate under an inert gas atmosphere. This can theoretically be accomplished by the use of a beefed up recycle system so that all secondary gas going into the furnace is recycle gas. In addition, a proportioning burner must be used which controls the primary air so as to quantitatively burn the gas without a surplus of oxygen. A third requirement is to maintain a slight positive pressure at all points in the system where leakage can occur so that leakage is outward rather than inward. The composition of gas which would be recycled in such a system is shown in table 1. As long as the temperature is held above 212° F., water vapor acts just like nitrogen.

In order to gain preliminary information as to whether inert gas dehydration might be beneficial in retaining xanthophylls, laboratory experiments were set up in which fresh alfalfa samples were dried at two temperatures, 221° F. and 239° F., and under a flowing stream of either nitrogen, carbon dioxide, or air. For a control value a sample was frozen with dry ice and then freeze dried without thawing. The data in table 2 show the results obtained with the nitrogen inert gas drying as compared with air drying and freeze drying. Carotene losses were substantial in the air-dried sample as were the losses of lutein--the main pigmenter

Table 1.--Composition of gases (percent)

Component	(a) Natural gas	(b) Air	(c) Quantitatively burned gas + air	(d) Recycle gas burner gases + evaporated water	(e) Inert gas used in gas storage (c & d dry basis)
Methane - - - -	90.0	---	---	---	---
Ethane - - - -	7.0	---	---	---	---
Propane - - - -	1.2	---	---	---	---
Other hydro-carbons - - - -	.3	---	---	---	---
CO_2 - - - - - - -	.5	---	8.3	3.9	12
Nitrogen - - -	1.3	78.37	63.4	28.9	88
Oxygen - - - - -	---	20.84	---	---	---
H_2O - - - - -	---	.79	28.0	67.1	---

Table 2.--Carotenoid content of alfalfa dried under nitrogen

Atmosphere	Drying temp.	Meal moisture	Carotene	Total xanthophyll	Nonepoxy xanthophyll
	°F.	Percent	Mg./lb.	Mg./lb.	Mg./lb.
Air dried - - - -	221	6.40	118.7	188.2	170.0
N_2 dried - - - -	221	7.81	158.5	238.0	215.3
Air dried - - - -	239	3.13	127.0	171.3	166.4
N_2 dried - - - -	239	3.09	146.5	220.2	213.0
Vacuum freeze-dried - - - -		6.22	156.3	368.0	217.0

of alfalfa. These losses were essentially eliminated by drying under nitrogen. In the case of total xanthophylls (X), N_2 drying reduced losses of X but did not eliminate them. This means that the epoxide xanthophylls are lost by other means than oxidation. This is not of practical importance since the epoxide xanthophylls are not effective pigmenters. Our interest is in the nonepoxide xanthophylls, especially lutein. Table 3 shows that carbon dioxide serves equally well as an inert gas and virtually eliminates losses of lutein and carotene.

Table 3.--Carotenoid content of alfalfa dried under carbon dioxide

Atmosphere	Drying temp.	Meal moisture	Carotene	Total xanthophyll	Nonepoxy xanthophyll
	°F.	Percent	Mg./lb.	Mg./lb.	Mg./lb.
Air dried - -	221	9.51	113.3	181.2	158.6
CO_2 dried - -	221	7.32	124.3	242.0	214.9
Air dried - -	239	4.31	124.4	174.2	164.5
CO_2 dried - -	239	4.96	156.7	213.5	195.6
Vacuum freeze-dried - - -		6.22	156.3	368.0	217.0

In our earlier work on xanthophyll stability during storage we found that freeze drying of alfalfa yielded a much more stable product than dehydration (1). This was attributed to retention of natural antioxidants in the freeze-dried alfalfa. Normal dehydration destroyed these antioxidants. We, therefore, set up the laboratory samples from the inert gas drying experiment described above in a stability test. The results are presented in table 4.

Table 4.--Carotenoid stability during storage of alfalfa dried under inert gases

Atmosphere	Drying temp.	Meal moisture	Carotene retention[2]	Total xanthophyll retention	Nonepoxy xanthophyll retention[2]
	°F.	Percent	Percent	Percent	Percent
Freeze-dried-	---	6.22	46	48	61
Air dried - -	221	9.51	62	54	52
N_2 dried - -	221	7.81	36	62	68
CO_2 dried - -	221	7.32	50	86	95
Air dried - -	239	4.31	36	54	54
N_2 dried - -	239	3.09	49	64	64
CO_2 dried - -	239	4.96	46	80	80

[1] Stored 12 weeks at 90°F.

[2] Percent of carotenoid present at start of storage.

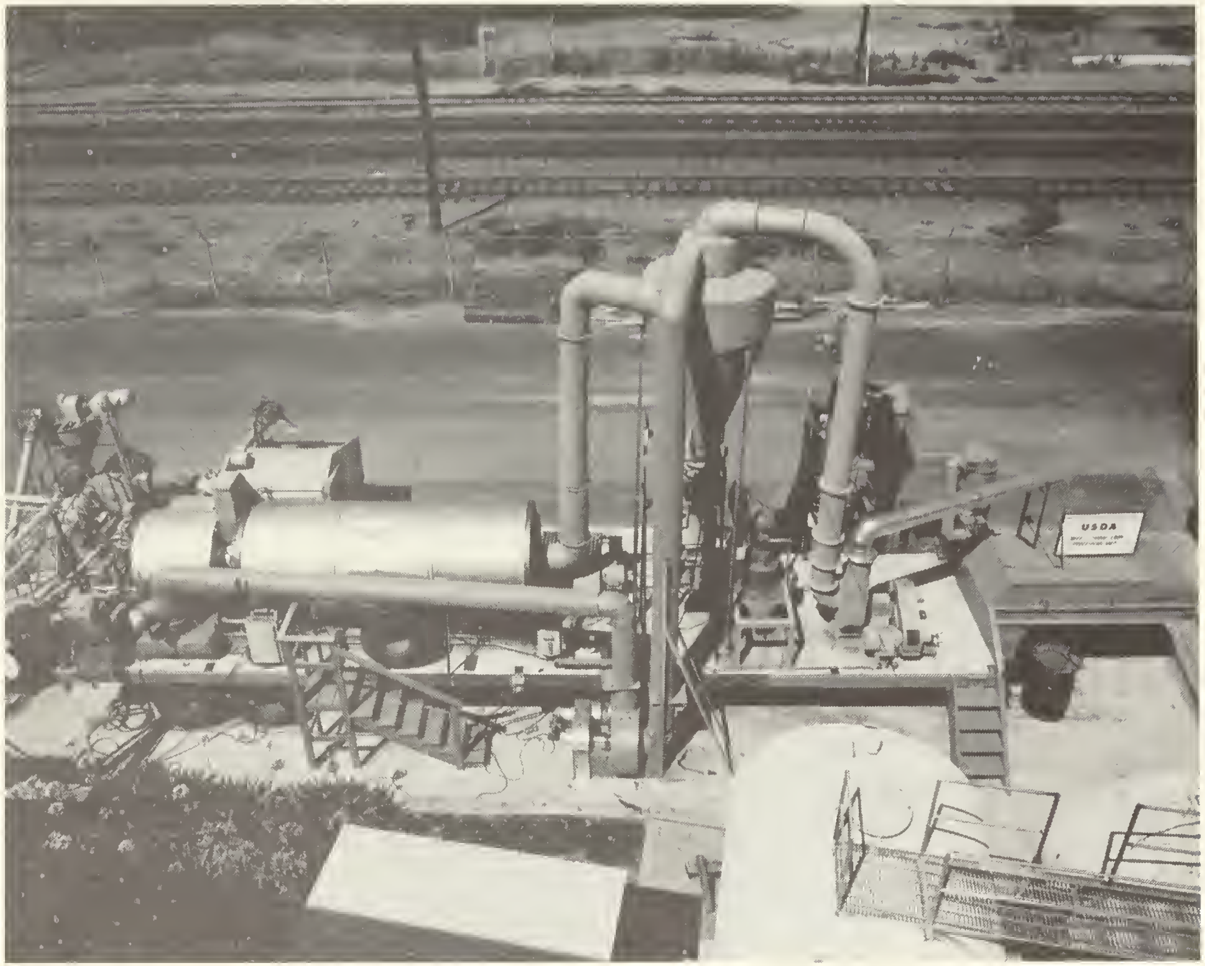

Figure 1.--Pilot dehydrator equipped for recycling.

The total as well as the nonepoxide xanthophyll in the meals dried under the inert gases was better retained during storage than the xanthophylls in either the air- or freeze-dried meals. This was due in part to the preservation of the natural antioxidants already present in the meals. However, there may be some enhancement of the natural antioxidant activity during inert gas drying. This is suggested by the better retention of the nonepoxide xanthophylls in the CO_2 and N_2 dried meals. The apparent higher rate of loss of total xanthophyll in the freeze-dried alfalfa is due to isomerization of the 5-6 epoxide xanthophylls to their 5-8 derivatives and subsequent shift in absorbance to shorter wave lengths which are not measured at 475 mμ.

This laboratory work has encouraged us to proceed with conversion of our pilot-plant dehydrator to a recycle system. The initial conversion is now completed and is shown in figure 1. A few experiments have been carried out and we can already see points for future changes. One point, which we have been able to demonstrate, is an auxiliary benefit to inert gas drying. In the first experiment we ran we found that when alfalfa was being dried at the same rate with the same rear end temperature, the front end temperature dropped from 1600° F. to about 1300° F. This drop we ascribe to the higher heat capacity of water vapor as compared with air (8.822 cal/mol vs. 6.998 cal/mol). This means that the same volume of water as gas when cooled from 1300° to 250° F. will dry as much alfalfa as air will when it is cooled by evaporation from 1600° to 250° F. The benefit to be obtained in existing plants is in the maintenance cost of the firebox or tube. If other design factors do not become limiting, it may mean that greater throughput could be obtained.

These matters will have to be settled by further research and development. Let it suffice to say that we are greatly encouraged by the possibilities for improvement of the dehydration process by stack gas recycling.

In closing let me simply list the possible benefits to be derived from this work.

1. Improved retention of carotene and xanthophyll.
2. Improved retention of natural antioxidants during dehydration to yield a more stable product.
3. Reduction of air pollution by incineration of part of the alfalfa dust which normally escapes from the cyclones.
4. Recovery of a part of the heat now wasted in the stack gases.
5. Operation of the front end of the dehydrator at lower temperatures.

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ALFALFA PRODUCTS FROM SEPARATION MILLING--AN ECONOMIC STUDY

Carl J. Vosloh, Jr., D. D. Kuzmicky, G. O. Kohler,
and R. V. Enochian[1]

Western Marketing and Nutrition Research Division,
Agricultural Research Service, USDA,
Berkeley, Calif.

During the late 1960's, personnel at the Western Regional Research Laboratory developed a technique for separating whole alfalfa chops. This method utilized controlled velocity air currents in a specially designed closed system apparatus to separate the light and heavy particles. The lighter particles are primarily leaf and the heavier material is essentially the stem portion of the plant.

This will provide two different products, each with specific advantages for feeding different classes of livestock. The lighter material is high in protein, vitamins, and xanthophyll and low in fiber, which makes it suitable for feeding poultry and swine. The heavier material, somewhat lower in protein and higher in fiber, is suited for feeding cattle.

The technique of "air separation" provides the alfalfa dehydrator with the opportunity to market "tailor-made" products. "Tailor-made" products would be designed to fit specific rations insofar as it is economically feasible.

The Economic Research Service is cooperating with the Agricultural Research Service's Western Regional Research Laboratory in an attempt to establish some economic values for the new dehydrated alfalfa products.

In this discussion, we will evaluate the imputed values of these "tailor-made" dehydrated alfalfa products and relate them to the added cost of separation. Parametric linear programming is used to obtain these imputed values. Parametric linear programming is a further development of linear programming. This technique enables the researchers to analyze, at different levels, the ingredient being studied. In a least-cost feed analysis, price is analyzed as the flexible item. The computer is programmed so the price of the key ingredient is lowered by given increments to arrive at a series of acceptance prices and quantities that would be used in a series of least-cost rations. The prices of other ingredients are held constant.

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[1] Respectively: Agricultural economist, Economic Research Service, Washington D. C., research chemist, and chief of Field Crops Laboratory, Western Marketing and Nutrition Research Division, Agricultural Research Service; and agricultural economist in charge, Economic Research Service, USDA, Berkeley, Calif.

This technique was developed and used in an earlier phase of this work. In 1968 Agricultural Economic Report No. 130, Alfalfa Meal in Poultry Feeds--An Economic Evaluation Using Parametric Linear Programming, was released by the U.S. Department of Agriculture. This was a pioneering study in evaluating a feed ingredient using this technique. An evaluation was made which used 20 percent dehydrated alfalfa in a broiler-finisher ration for one time period and in one market. This study brought out that the most important points of substitution of dehydrated alfalfa for other ingredients were related to needs for xanthophyll, Vitamin A, and tryptophan in the ration. Higher protein dehydrated alfalfa products are relatively inexpensive sources of these nutrients.

At the present time, we are evaluating the lower protein, higher fiber fraction obtained through separation as well as making a more comprehensive analysis of the high-protein fraction. The protein level of the low-protein fraction could vary between 13 and 15 percent. In our analysis, we are using a 13-percent product and comparing its value and use to a standard 17-percent protein dehy. Table 1 contains the nutritional values for 13- and 17-percent products in ruminant feeds. Table 2 contains the nutritional values for 17- and 22-percent products in poultry and swine feeds.

Table 1.--Nutritional values of dehydrated alfalfa products in ruminant feeds

Item	Dehy with protein content of	
	13 percent	17 percent
	Percent	Percent
Total digestible nutrients	51.0	56.0
Total protein	13.0	17.0
Digestible protein	9.7	12.7
Fat	1.5	2.3
Calcium	1.22	1.39
Phosphorus	.24	.26
Fiber	32.1	27.8

Table 2.--Nutritional values of dehydrated alfalfa products in poultry and swine feeds

Item		Dehy with protein content of	
		17 percent	22 percent
Metabolizable energy	kcal./lb.	750.00	800.00
Protein	percent	17.00	22.00
Arginine	ditto	.75	1.01
Glycine	ditto	.88	1.21
Isoleucine	ditto	.84	1.16
Lysine	ditto	.73	1.03
Methionine	ditto	.28	.38
Methionine and cystine	ditto	.46	.62
Threonine	ditto	.75	1.05
Tryptophan	ditto	.45	.55
Phosphorus	ditto	.23	.28
Calcium	ditto	1.30	1.47
Fiber	ditto	24.30	18.50
Xanthophyll	mg./lb.	117.00	182.00

The 13-percent fraction is being evaluated in a beef finisher ration with roughage. Comparison with the standard 17-percent protein dehy is made at two locations: Los Angeles and Tri-Cities (Rock Island, Davenport, and Moline). Comparison is also made for two time periods: November 1966-January 1967 and August 1968-October 1968. Our analyses will reflect the effects of the major factors on prices. These factors are protein content, feeding location, and time.

For this discussion we are analyzing the values of both fractions obtained through air separation. We are using the imputed values for selected dehydrated alfalfa products obtained through the parametric linear programming technique. Assume a dehydrator could harvest a 17-percent protein alfalfa chop. He could separate this into both a 13- and a 22-percent protein product. About 55 percent of the total volume would be the 13-percent fraction and the remaining 45 percent the 22-percent protein material. With this basic assumption in mind, we are proposing that if separation is made the 13-percent protein dehy will go into ruminant feeding and the 22-percent product will go to feeding poultry and swine. For comparison purposes, we also are assuming that a straight 17-percent protein product could be fed to all classes of livestock.

As mentioned previously, a beef finisher ration with roughage was used to develop values for the 13-percent protein material and a layer light breed ration has been selected for the 22-percent protein dehy. Tables 3 and 4 contain the constraints placed on the ruminant and poultry matrices.

Table 3.--Nutrient restrictions for beef finisher ration with roughage

Nutrient restriction		Quantity
Total digestible nutrients	percent min	75.00
Total protein	ditto	11.00
Digestible protein	ditto	8.25
Nonprotein nitrogen	percent max	.64
Total fat	ditto	100.00
Calcium	percent min	.25
Phosphorus	ditto	.20
Salt	percent eq.	.25
Fiber	percent max	100.00
Dry matter	percent min	--
Molasses	ditto	2.50
Molasses, cane	percent max	10.00
Wheat mids	ditto	20.00
Wheat bran	ditto	10.00
Soybean millfeed	ditto	20.00
Rice bran	ditto	20.00
Brewers dried grains	ditto	20.00
Corn gluten feed	ditto	20.00
Distillers dried grains	ditto	20.00
Malt sprouts, dried	ditto	20.00
Animal fat	ditto	5.00
Dehy alfalfa (17 percent)	ditto	100.00
Beet pulp	ditto	20.00
Corncobs	ditto	5.00
Cottonseed hulls	ditto	5.00
Calcium over minimum	ditto	1.00
Phosphorus over minimum	ditto	1.00
Roughage dry hundredweight over minimum	percent	10.00

Table 4.--Nutrient restriction for layer light breed ration

Nutrient restriction		Quantity
Metabolizable energy	kcal./lb min	1350.00
Protein	percent min.	--
Arginine	ditto.	.800
Glycine	ditto.	.600
Isoleucine	ditto.	.500
Lysine	ditto.	.500
Methionine	ditto.	.280
Methionine and cystine	ditto.	.530
Threonine	ditto.	.400
Tryptophan	ditto.	.150
Available phosphorus	ditto.	.300
Calcium	ditto.	3.250
Added fat	percent max.	10.00
Fiber	ditto.	100.00
Xanthophyll	mg./lb min	7.00
Cottonseed meal solvent 41 percent	percent max.	7.5
Meat meal 55 percent	ditto.	5.0
Meat and bonemeal 50 percent	ditto.	5.0
Feather meal 85 percent	ditto.	2.5
Poultry by-products 55 percent	ditto.	5.0
Rice bran	ditto.	5.0
Calcium over minimum	ditto.	.75

Table 5 shows some of the preliminary values obtained thus far. The Los Angeles market tended to establish a higher value for these dehydrated alfalfa products than did the Tri-Cities area. In table 5, we see that in November 1966-January 1967 the 13-percent product in the Los Angeles market was valued at $40.80 a ton. The 17-percent was valued somewhat higher at $48.80 a ton. At these prices, between 9 and 10 percent of the beef-finisher ration would be made up of dehydrated alfalfa. In the second time period, the 13-percent product would be incorporated into the ration at about the 6-percent level with a value of $35.40 a ton. Dehy 17-percent would have a value of $39.40 a ton and would make up about 15 percent of the ration.

Table 5.--Summary of parametric linear programming value analyses for selected dehydrated alfalfa products: By location, type of feed, and product for selected time periods

Item	Nov. 1966 - Jan. 1967		Aug. 1968 - Oct. 1968	
	Value per ton	Percent in ration	Value per ton	Percent in ration
	Dollars	Percent	Dollars	Percent
Los Angeles:				
Beef finisher:				
13-percent dehy	40.80	10.00	35.40	5.94
17-percent dehy	48.80	9.04	39.40	15.38
Layer:				
17-percent dehy	102.80	1.14	95.00	.79
22-percent dehy	151.60	.81	134.80	.63
Tri-Cities:				
Beef finisher:				
13-percent dehy	34.80	5.00	33.40	5.00
17-percent dehy	39.80	5.00	37.00	5.00
Layer:				
17-percent dehy	57.80	.55	62.20	.61
22-percent dehy	78.40	.50	88.60	.50

In the Tri-Cities market area, the 13-percent dehydrated alfalfa had a value of $34.80 a ton (table 5). During the same period, 17-percent would have been worth about $39.80 a ton. At these prices, the beef-finisher rations would have taken 5 percent of either the 13- or 17-percent dehy. Ingredient prices tended to be lower in the 1968 time period. Therefore, both 13- and 17-percent dehy were valued lower than in the earlier period. The 13-percent product established an imputed value of $33.40 a ton, and the 17-percent dehy was $37 a ton.

In evaluating the 22-percent fraction, we have found this product to be worth considerably more than the high-fiber material. During the first time period, the 22-percent product was valued at $151.60 a ton in the Los Angeles market. However, in the same time period its value was $78.40 in the Tri-Cities market. In the second time period, the value in the Los Angeles market had dropped to $134.80. In the Tri-Cities area the value of this product increased to $88.60.

It is interesting to note that the 17-percent dehy commanded a higher value when used in the layer ration than when it was used in the beef-finisher ration. This demonstrates very clearly the reason for separation. Nutritional elements such as xanthophyll are more essential in poultry feeds than in ruminant feeds. Values in layer rations in Los Angeles ranged from $102.80 in the first time period down to $95 in the second time period. However, in the Tri-Cities market the imputed value of the 17-percent material increased from $57.80 a ton up to $62.20 during this time span.

Other values were established on value curves for the dehydrated alfalfa products. However, only the value-quantities in table 5 are used in this discussion. A more detailed interpretation will be made using these data in a report that will be published in the near future.

In table 6, we have developed a blended value for the two fractions: 13- and 22-percent protein dehydrated alfalfa. The values have been weighted by the weight distribution which can be obtained when whole dehydrated alfalfa (17-percent protein) is air separated. In the first time period, blend value in Los Angeles would be about 65 percent greater than in the Tri-Cities area. In the second time period, value in Los Angeles would be 35 percent larger.

Gross margins have been derived from the imputed values in table 5. Gross margins were calculated by the following method: The 17-percent dehy value is assumed to be the base value. Margins between alfalfa product values are taken for each feed for a particular time period. The margin for each feed is weighted by applying the percentage breakdown each of the product is of the total dehy product. That is, value of 13-percent product is weighted by 55 percent and of 22-percent is weighted by 45 percent, and the two will provide the blended value per ton.

Table 6.--Value analyses of separated dehydrated alfalfa products related to nonseparated dehydrated alfalfa
[Values are dollars per ton]

Market and time period	Separated products: Value 13-percent dehy[1]	Separated products: Value 22-percent dehy[1]	Blended value[2]	Gross margin[3]
Los Angeles:				
Nov. 1966 - Jan. 1967	40.80	151.60	90.60	17.56
Aug. 1968 - Oct. 1968	35.40	134.80	80.00	15.71
Tri-Cities:				
Nov. 1966 - Jan. 1967	34.80	78.40	54.40	6.52
Aug. 1968 - Oct. 1968	33.40	88.60	58.20	9.90

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[1] From table 5.

[2] Blended value developed from percentage each product would be of the total: 55 percent would be 13-percent protein dehy and 45 percent would be 22-percent protein dehy.

[3] Gross margins are derived from values of 17-percent dehy in table 5.

Gross margins range from a high of $17.56 a ton in Los Angeles during the first time period to $6.52 a ton in Tri-Cities during the same period. In both time periods the margin in the Tri-Cities area is considerably below that in Los Angeles. During the second time period, the margin increased in the Tri-Cities and decreased in Los Angles but was still higher in Los Angeles.

We may evaluate the imputed values with another comparison (table 7). A comparison may be made using the actual market prices for 17-percent dehydrated alfalfa. The method of attaining a blended value is the same as described above.

Table 7.--Value analyses of separated products to 17-percent dehydrated alfalfa
[Values are dollars per ton]

Market and time period	Value 13-percent dehy[1]	Value 22-percent dehy[1]	Blended value[2]	Market price 17-percent dehy	Gross margins
Los Angeles:					
Nov. 1966 - Jan. 1967- -	40.80	151.60	90.60	53.40	37.20
Aug. 1968 - Oct. 1968- -	35.40	134.80	80.00	55.20	24.80
Tri-Cities:					
Nov. 1966 - Jan. 1967- -	34.80	78.40	54.40	68.20	-13.80
Aug. 1968 - Oct. 1968- -	33.40	88.60	58.20	41.60	16.60

[1] From Table 5.

[2] Footnote 2, table 6.

The gross margins are quite different. They range from a high of $37.20 a ton in the first time period in the Los Angeles market to a low of -$13.80 in the first time period in the Tri-Cities market. Gross margins as calculated for this discussion show that margins can vary considerably depending upon the basic price used.

When the individual dehydrator would estimate a gross margin representative of his situation, he may use a much different set of weighting factors. His factors would be quite different depending on whether his primary market was poultry or beef. In this situation transportation cost may have a great deal to do with his decision to separate dehy.

The important question remains, "Is this margin or differential enough to justify separation?"

In May 1970, USDA released Marketing Economic Report No. 881, Alfalfa Dehydration, Separation, and Storage: Cost and Capital Requirements. In this study, we attempted to synthesize the additional costs incurred by an alfalfa dehydrator using the "air separation" method.

Table 8 provides specifications with regard to sizes of dehydrated plant models in our analyses. Basic input and output data used in these models were typical for the Kansas and Nebraska area. Therefore, costs would be more representative of the Midwestern dehydrator costs than for a dehydrator on either the west coast or in the East. Many factors influence both production and operating costs for each locality.

Table 9 summarizes the cost estimates developed from this analysis. Dehydration costs were synthesized for basic models without separation. Three alternative flow systems using separation equipment were evaluated and compared with the basic model's costs.

Alternative I does not include grinding equipment since the material is passed through a transfer fan. Leaves are fragile at this point and are shattered. This fan provides adequate differential grinding action to separate the leaves from the stems. After the fractions are separated, each is deposited in a meal bin over the pellet mill.

Alternative II has the same basic equipment in the receiving, dehydrating, and grinding operations as the basic model. The separation process is added to the system following the grinding, and the remaining equipment is essentially the same as in alternative I.

In alternative III, the receiving, dehydrating, and separating equipment is similar to that used in alternative I. From the separator, the fine fraction moves to the pellet mill, and the coarse fraction is diverted to the grinder. The coarse fraction is ground and then moved to the pelleting operation.

The cost of separation ranged from $3.24 a ton for alternative II in the smaller plant producing about 5,000 tons a year to 30 cents per ton for alternative I in the 17,300-ton plant (table 9). In relating these additional costs to margins in table 6, it would appear that all model plants would find it economically feasible to separate the alfalfa chop.

We are assuming the separation costs in table 9 are appropriate to both markets and that the alfalfa dehydrator could sell his products for the established values. If the dehydrator could increase output beyond our tonnage estimates, he could compete more effectively since his per-ton costs should be decreased.

Table 8.--Specifications of model alfalfa dehydration and separation plants

Model[1]	Evaporating capacity per hour	Drum	Rated capacity per hour	Hourly production	Annual production[2]
	Pounds		Tons	Tons	Tons
A	10,000	Single-pass	1 to 2.5	1-1/2	4,950
B	12,000	Triple-pass	1 to 2.5	1-3/4	5,775
C	18,000	Single-pass	2 to 4	2-3/4	9,075
D	22,000	Triple-pass	2 to 4	3-1/2	11,550
E	30,000	Single-pass	4 to 6	4-1/2	14,850
F	33,000	Triple-pass	4 to 6	5-1/4	17,325

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[1] The 6 volume size plants are designated by letter (A, B, C, etc.).

[2] Operating for 22 hours a day, 150 days a year.

Table 9.--Comparison of fixed, variable, and total costs per ton for alfalfa dehydration in model plants with and without separation
[Values are dollars per ton]

Model and cost item	Method of operation			
	No separation	Separation		
	Basic model	Alternative I	Alternative II	Alternative III
Model A:				
Fixed.	6.01	7.59	7.92	7.92
Variable	12.36	12.48	13.69	13.18
Total.	18.37	20.07	21.61	21.10
Difference . . .	--	1.70	3.24	2.73
Model B:				
Fixed.	5.49	6.84	7.13	7.12
Variable	11.61	11.58	12.63	12.19
Total.	17.10	18.42	19.76	19.31
Difference . . .	--	1.32	2.66	2.21
Model C:				
Fixed.	3.96	4.81	5.03	5.00
Variable	9.92	9.97	10.75	10.39
Total.	13.88	14.78	15.78	15.39
Difference . . .	--	.90	1.90	1.51
Model D:				
Fixed.	3.32	4.00	4.15	4.14
Variable	9.29	9.35	10.06	9.74
Total.	12.61	13.35	14.21	13.88
Difference . . .	--	.74	1.60	1.27
Model E:				
Fixed.	2.86	3.39	3.52	3.52
Variable	8.72	8.67	9.22	8.98
Total.	11.58	12.06	12.74	12.50
Difference . . .	--	.48	1.16	.92
Model F:				
Fixed.	2.75	3.20	3.33	3.31
Variable	8.26	8.11	8.71	8.41
Total.	11.01	11.31	12.04	11.72
Difference . . .	--	.30	1.03	.71

The price relationships in this study were established through nutritional benefits. Certain benefits or factors are difficult to estimate in dollars and cents. Palatability is a good example. How does one establish a palatability factor? This is a major problem and will no doubt receive even more attention in the near future than in the past.

There are numerous new developments in ruminant nutrition which are having or will have a decided effect on the use of dehydrated alfalfa in ruminant rations. Changes having the greatest influence on dehydrated alfalfa use are: (1) Development of high-urea supplements; and (2) the emphasis on high-energy rations for cattle feeding.

Research has shown that dehydrated alfalfa meal contains certain unidentified growth factors which affect the performance of beef cattle on certain feeding programs. This is especially true when high-energy diets are fed and high levels of urea are used to substitute for natural protein. Researchers have provided evidence that dehy alfalfa significantly improves protein synthesis from nonprotein nitrogen in certain ruminant feeds.

In conclusion, air separation of alfalfa chop appears to be economically feasible. Parametric linear programming has made it possible to obtain intrinsic value curves. The values obtained in this research have indicated several important factors. Values of an ingredient are dependent on the competitive environment in which it is used.

The effects of ingredient nutrient content, ration specifications, geographical location reflecting ingredient availability, and prices were examined in this analysis. Changes in any of these factors influence the location of the points of substitution on the intrinsic value curves. For example, if unidentified growth factors in dehydrated alfalfa can be given values, the intrinsic value curves will no doubt change to some degree.

Air separation is not the answer to all the alfalfa dehydrator's problems. However, it may help to improve his position in the market. Commercial dehydrators will differ in their demands. Air separation can remove some of the uncertainties of the dehydrator such as: Being able to supply the market with the quantities of higher quality products regardless of weather hazards.

Commercial dehydrators must be able to answer the one important question: Is there a market for these "tailor-made" products? There is always the possibility that a market could be established if feed manufacturers could be guaranteed a supply throughout the year.

Separation makes it possible for the dehydrator to make more efficient use of his harvested crop. Air separation will help to eliminate protein "give aways." If the protein quality of an alfalfa chop is higher than the market wants, the dehydrator can remove a portion of the protein and blend this with a lower protein quality product. He can "tailor-make" his output to suit the particular market demand. At this point in time, separation may not be economically feasible for all alfalfa dehydrators. However, as the feed manufacturer becomes more aware of the need for uniform quality of ingredients in formulation of feeds the greater will be the demand for these dehydrated alfalfa products.

PROCESSING CELLULOSIC WASTES FOR FEED

H. G. Walker, Jr. and D. D. Kuzmicky

Western Marketing and Nutrition Research Division,
Agricultural Research Service, U.S. Department
of Agriculture, Berkeley, Calif.

At this laboratory, we have been attempting to process cellulosic wastes like straws and sugarcane bagasse into animal feeds. As you know, these materials have different feed characteristics from those of a high-quality forage like alfalfa. Even though they are ordinarily considered worthless enough to burn, they can serve as energy sources for ruminant animals because they contain cellulose and hemicelluloses digestible by rumen micro-organisms. In the West, since burning rice straw in California and grass straw in Oregon are about to be outlawed, development of alternative disposal procedures is a real necessity.

It has been known for a long time that some treatment of these waste cellulosic products improved their digestibility. Over a 100 years ago German scientists first began working on the problem. This early work has been reviewed in 1924 by Archibald (1). In 1891 Lehmann (6) treated rye straw with sodium hydroxide and found that the straw cellulose produced was nearly equal in feed value to that of soluble carbohydrates. Archibald states that in 1899, Kellner, a famous German animal scientist, reported that rye straw hydrolyzed by the process used in paper manufacture had a digestibility of 88 percent and was capable of producing more fat in ruminants than pure potato starch (1). Throughout the early 1900's, up to World War I, many researchers studied various upgrading procedures using sodium hydroxide, lime, sodium, carbonate, and other chemicals at boiling temperatures and under pressure. A number of these processes were used in Europe during the war years when feed was in short supply. In 1919, Ernst Beckmann (2) made an important breakthrough on a process for treatment of straws with NaOH at ambient temperatures. According to Archibald (1) this process was so much simpler and more economical that it rapidly superseded those already in use and became the subject of careful investigation by German experiment stations and others. Archibald, in the U.S. (1), for example studied its application on oat hulls, barley hulls, rice hulls, cottonseed hulls, and flax shives and found it useful.

Table 1.--Comparison of digestibility of unprocessed straws and straws processed by 2 methods

Straw	DMD - Enzymatic (percent)		
	Control	Steam[1]	NaOH (3 percent)[1]
Rye grass	40	49	76
Barley	37	56	83
Wheat	37	47	75
Rice	34	43	61
Alfalfa	52	61	81

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[1] Liquid:solids = 2:1. Processed at 231°C. for 3 minutes.

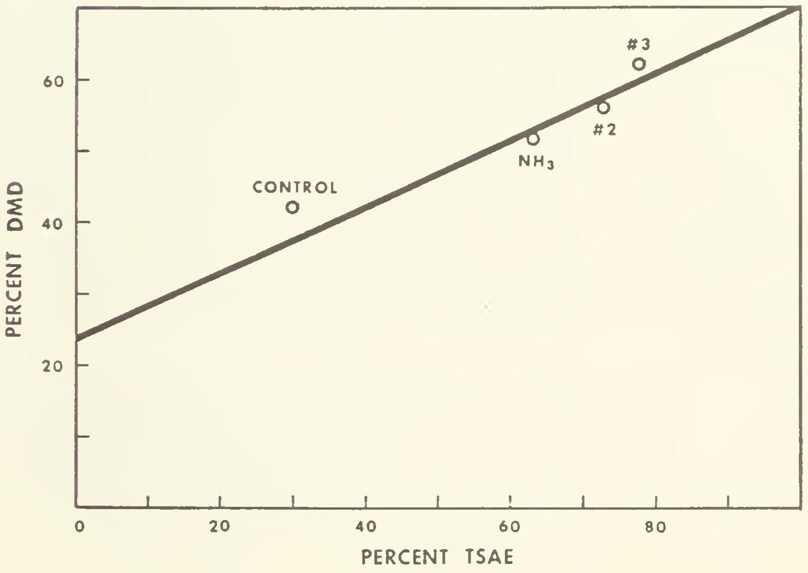

Figure 1.--Comparison of results by TSAE vs. Davis DMD (sheep) method.

Almost all processing involves wetting the straw, so that if the product is not to be consumed immediately at the feedlot it must be dehydrated.

This now leads us directly to the alfalfa dehydrating industry and the large number of Midwestern alfalfa dehydrating plants that operate for less than 6 months a year. If these plants could be used for 12 months of the year, utilization of land, work crews, equipment, buildings, etc. could be considerably more efficient.

What I propose to do is to explore the possibility of processing cereal straw for cattle feed at the dehydrator in the off-season for alfalfa operations. In Kansas and Nebraska alone there are over 25 million tons of wheat straw potentially available for feed use and plenty of cattle to consume it.

Results in table 1 indicate that it is possible to increase the in vitro digestibility of straws by processing (3). Figure 1 compares results from actual sheep-feeding experiments with enzymatic data on some rice straw samples processed here and tested at the University of California at Davis (4). Correlation between the two methods of testing is good. There is no question but that we can improve digestibility of straw, but there is a question of whether processed and redried straw could compete as a feedstuff in the grain-rich Northern Plains States where most of the dehydrators are. We have looked at this problem using the same Parametric Linear Program (PLP) techniques and data that Mr. Vosloh reported in his study on the economics of separation milling (p.136). Estimates are based on prices for the Tri-Cities area (Moline, Davenport, and Rock Island) for a period covering November 1966 through January 1967 when grain and other ingredient prices were high and August through October 1968 when prices were low. Wheat straw at three different TDN levels (40, 60, and 70 percent) was evaluated in two rations; a beef finisher with roughage and a dairy ration with roughage.

Table 2 shows a typical ration. This happens to be a least-cost fattening ration formulated with and without the three straws. Note that untreated straw enters the formula only as roughage, replacing hay, but that processed straw, on the other hand, competes with grain as an energy source.

Table 2.--Beef-fattening ration with roughage
(High-price period)

Ingredient	Percent in ration			
Ground corn	--	23.5	33.2	5.4
Steam rolled milo	65.9	42.2	12.3	37.6
Cane molasses	10.0	10.0	10.0	10.0
Alfalfa hay 15 percent	20.0	5.4	20.0	20.0
Dehy alfalfa 17 percent	2.5	2.5	2.5	2.5
Urea	.5	1.1	1.0	1.0
Calcium carbonate	.3	--	--	--
Salt	.25	.25	.25	.25
Trace nutrient mix	.5	.5	.5	.5
Wheat straw 40	--	14.6	--	--
Wheat straw 60	--	--	20.3	--
Wheat straw 70	--	--	--	22.7

As for the value of the straw products, PLP results at the low-price period are shown in table 3. The points shown are not necessarily where straw first entered the ration, but are, instead, the first where fairly substantial quantities might be used in the diet.

Untreated straw is valued at about $21/ton when 10 percent or more is used; treated straw appears to be worth about $38 at reasonable usage levels.

In table 4 we see that in the high-price period, untreated straw is worth less than $20/ton but that treated straw is worth up to $45/ton in some cases.

These data indicate that processed straw could have potential feedstuff value in the Midwest, but how much would it cost to produce it? Good cost data are available because we don't even know the exact process to use. However, we can speculate a little on the subject anyway. An average dehydrating plant might operate as follows: (5, 9):

1. Produces 3 tons of dry alfalfa per hour or 6 tons of dry straw product per hour.

2. Operates 16 hours per day for 150 days.

3. Produces 14,400 tons of straw product per season.

Table 3.--Low-price period

Ration	Wheat 40		Wheat 60		Wheat 70	
	Percent in ration	Value dollar/ ton	Percent in ration	Value dollar/ ton	Percent in ration	Value dollar/ ton
Dairy with roughage	10.8	22.00	5.7	39.00	5.7	39.00
	21.0	21.40	14.5	38.20	14.5	38.20
	--	--	30.0	32.00	36.6	33.20
Beef finisher with roughage	14.2	21.33	10.7	38.07	24.4	38.54
	20.0	15.81	16.6	32.38	38.4	34.53

Table 4.--High-price period

Ration	Wheat 40		Wheat 60		Wheat 70	
	Percent in ration	Value dollar/ ton	Percent in ration	Value dollar/ ton	Percent in ration	Value dollar/ ton
Dairy with roughage	4.3	24.12	5.3	45.40	23.0	45.40
	17.7	19.66	27.2	41.78	33.5	44.6
Beef finisher with roughage	14.6	15.72	20.3	38.63	8.2	45.18
	20.0	15.38	37.6	35.55	22.7	44.83
	--	--	--	--	57.2	42.00

We would like to assume further that the alfalfa operation would pay all the yearly fixed costs except those directly chargeable to new equipment obtained to process straw.

As far as raw material is concerned, straw could be harvested in the summer and stacked at the roadside for transportation to the plant during the winter as needed. If the straw is baled, the delivered cost at the factory might be as high as $15/ton. This includes a baling fee, a hauling fee, and some profit margin for the grower. Improved bulk hay handling processes should reduce this cost somewhat. Material and transportation costs do present a real problem for a successful operation.

Process costs would depend on the type of treatment chosen. It an ambient temperature, long-term Beckmann-type process were used, a modest capital outlay would be required for silo facilities, presumably of the pit-type. Variable costs would be minimal for labor, utilities, and maintenance. There would be a probable chemical cost of about $3/ton if sodium hydroxide were used at the 4-percent level. Dehydration cost would probably be less than that for alfalfa because of lower initial moisture and lack of need for an antioxidant, maybe $7 to $8/ton. Overall costs for this type of operation would be less than $30/ton.

If a high-pressure alkali treatment system were built, overall process costs would be somewhat higher because of the greater initial expenditures for equipment. However, even when we considered a fairly expensive model, total costs including material were less than $33/ton.

Remembering that PLP values for treated straw ranged from $38 to $45/ton, the economics of processing straw at the dehydrator at less than $30 to $33/ton appears to be favorable at some periods of time. Obviously more processing and nutritional research, along with much more vigorous economic studies, will be required to answer the question as to whether the operation will be profitable. At the present time we think the outlook is quite favorable.

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